PROTEIN DEGRADATION DURING DIAUXIC GROWTH OF ESCHERICHIA COLI

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The phenomenon of diauxic growth was first demonstrated by Monod (1945). He grew E. coli in a medium containing both glucose and lactose, and found that there were two phases of growth separated by a lag period. A mechanism proposed to explain this is that the cells first utilised the glucose: during this period an intermediary metabolite was produced which repressed the synthesis of the enzymes necessary for lactose utilisation, even in the presence of lactose. When the glucose was exhausted, the cells had neither the enzymes necessary for lactose utilisation, nor amino acids for their synthesis. Then amino acids were derived from intracellular breakdown of protein (this increasing in rate when growth stopped) and used for the synthesis of proteins which included the now derepressed enzymes necessary for lactose utilisation. Exponential growth was then resumed (Mandelstam, 1963).

A similar growth lag has also been observed in other systems where cells were transferred to a minimal medium from one containing growth factors which they had the genotypic, but not phenotypic, ability to synthesise; e.g. arginine (Gorini, Gundersen and Burger, 1961), cysteine and methionine (Bourgeois, Wiame and Lelouchier-Dagnelie, 1960); or the complex mixture of amino acids and growth factors present in a broth medium (Maaløe, 1960).

Direct confirmation of the connection between diauxic growth and protein degradation has been obtained during growth of E. coli on a mixture of glucose and either lactate or glycerol.

Materials and Methods. E. Coli ML 328c lac^+leu^- was grown in a synthetic medium (Mandelstam, 1960), supplemented with DL-leucine (300 µg/ml), and containing the carbon sources and different concentrations of L-valine described below. All incubations were at 35° C.

Protein degradation was measured by labelling cells with radioactive L-valine, and then following its liberation into the culture medium. At the beginning of the experiment, culture samples were extracted with 5% trichloroacetic acid (TCA) at 90° C for 30 min. The protein precipitates were filtered on Oxoid membrane filters and washed successively with 5% TCA containing L-valine (150 µg/ml), 5% TCA, and 1% acetic acid. They were then glued to aluminium planchettes and their radioactivity determined, giving a measure of the original radioactivity of the cell protein. Culture samples were taken during the experiment, cooled to 0° C to prevent further metabolism, and centrifuged. The supernatant was removed, diluted, and its amino acids adsorbed onto columns of Zeo-Karb 225 in the H^+ form. The columns were washed through with water to remove inorganic salts, and the amino acids eluted with an excess of 2 N NH_4OH. The solutions were evaporated to dryness at 100° C, and the residues dissolved in water and transferred to aluminium planchettes. These were dried and their radioactivity determined, giving a measure of the amount of radioactive L-valine in the culture supernatant. Comparison of this with the radioactivity of the cell protein gave an estimate of protein degradation.

Preliminary experiments showed that radioactive L-valine was incorporated only into L-valine in the cell protein, and that cell lysis followed by extracellular protein degradation did not occur under the conditions used in these experiments. The liberation of intracellular

β-galactosidase into the culture medium was used as an index of cell lysis.

Corrections were made for loss of radioactivity during preparation of the samples, and for their self-absorption during determination of their radioactivity (in an Automatic Sample Changer Counter with a thin end-window Geiger tube detector).

Results. Cells were grown from a small inoculum in the synthetic medium containing glucose (1 mg/ml) and L-[^{14}C] valine (35 μg/ml; 0.1 μc/ml). They were harvested during exponential growth, washed twice in a medium containing L-valine (150 μg/ml) but no carbon source, and resuspended in a medium containing glucose (150 μg/ml) and L-valine (200 μg/ml). The latter was included to prevent reincorporation of radioactive L-valine liberated by protein degradation.

This culture was divided into six parts ((a) to (f)), and samples taken from each over a period of 90 min. for the determination of culture density and protein degradation. At this point the glucose was almost exhausted, and the following additions were made:

to culture (a) sodium lactate (800 μg/ml)
" (b) glycerol (800 μg/ml)
" (c) no addition
" (d) glucose (800 μg/ml)
" (e) glucose (800 μg/ml) and sodium lactate (800 μg/ml)
" (f) glucose (800 μg/ml) and glycerol (800 μg/ml).

The additions were delayed to ensure minimal synthesis of the enzymes necessary for the utilisation of lactate or glycerol during the first growth period.

Samples were taken for the determination of culture density and protein degradation for a further 150 min.

The results are shown in Fig. 1. Those of experiments (d), (e),

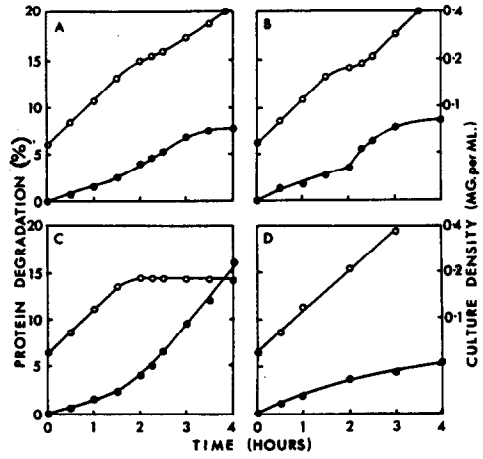

Fig. 1. Protein degradation during growth on: A, glucose (150 µg/ml) and lactate (800 µg/ml); B, glucose (150 µg/ml) and glycerol (800 µg/ml); C, glucose (150 µg/ml); D, glucose (950 µg/ml) alone, or with lactate (800 µg/ml), or glycerol (800 µg/ml). Symbols: ●, protein degradation; o, culture density.

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and (f) were similar, and are shown in Fig. 1, D. This indicated that neither lactate nor glycerol per se affected the rate of protein degradation.

Comparison of protein degradation and cell growth in experiments (c) and (d) shows that the rate of protein degradation in cells growing exponentially was slow (about 0.6% per hour), but increased rapidly (to about 6% per hour) when the glucose was exhausted.

When a second carbon source was present, in experiments (a) and (b), there was a diauxic lag of about 30 min. between the two periods of exponential growth. During this lag period, protein was degraded at an increased rate, whereas both before and afterwards it was degraded at the slow rate characteristic of growing cells.

These results support the suggestion that during a diauxic lag intracellular protein degradation supplies the amino acids for protein synthesis and that protein degradation is therefore an essential part of the mechanism of diauxic growth.

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