SYNTHESIS OF ENZYMES OF THE LACTOSE OPERON DURING DIAUXIC GROWTH OF ESCHERICHIA COLI

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The synthesis of inducible enzymes is generally considered to be regulated by two independent factors, (i) the highly specific intervention of a small molecule, the inducer, and (ii) a non-specific effect of the metabolic state of the cell referred to as catabolite repression. This model implies that the highest possible differential rate of synthesis of an enzyme occurs when saturating concentrations of a good inducer are used under conditions of minimum catabolite repression. Recently it has been reported that unusually high differential rates of synthesis of two enzymes of the lactose operon occur during part of the diauxic phase of a culture growing on a mixture of glucose and lactose (Attardi et al., 1963; Naono et al., 1965), implying that some mechanism exists to raise the differential rate of synthesis of these enzymes above the rate that has heretofore been considered the maximum attainable. The importance of such a mechanism led us to a reinvestigation of this question. The experiments reported here show that enzyme sunthesis during diauxie is not unusually high. The observed changes can be explained in terms of inducer and catabolite repression effects.

In the present study we have compared the differential rate of β-galactosidase synthesis during diauxie to the rate of synthesis during growth on glycerol and induction with isopropyl-β-D-thiogalactoside (IPTG), a condi-

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tion representing good induction and little catabolite repression. Protein synthesis was measured by the incorporation of a radioactive amino acid added at the onset of diauxie. This technique is less likely to lead to errors than adding the labeled amino acid one generation time before the onset of diauxie (Naono et al., 1965), since in the latter case one must determine the rate of a small increase in the large amount of radioactivity already incorporated before the onset of diauxie. Further, since the rate of incorporation of radioactivity measures the difference between the increase in radioactivity due to new protein synthesis and the loss of radioactivity due to the breakdown of previously synthesized protein, adding label at the onset of diauxie avoids errors due to turnover of previously synthesized protein, which would lead to a falsely low measure of new protein synthesis.

Protein synthesis in these experiments was measured by the incorporation of C^{14} l-isoleucine, an amino acid less likely to be incorporated into non-protein macromolecules than is dl-phenylalanine which was used previously. The results of a typical experiment are shown in Figure 1. There is a period lasting approximately 20 minutes during which the differential rate of β-galactosidase synthesis is much higher than at a later time, a result in agreement with the previous findings, but a comparison of the absolute rates of enzyme synthesis during diauxie with the rates in cells growing on lactose, or growing on glycerol and induced with IPTG shows that the rates of synthesis during diauxie are not unusual. Table I gives the results of a typical experiment in which the rate of β-galactosidase synthesis early in diauxie was found to approximate 80% of the rate characteristic of glycerol-grown cells induced with IPTG. Significant variations in the differential rate of β-galactosidase synthesis in diauxie have been observed; the ratio of the rate of synthesis early in diauxie to that on glycerol-IPTG ranged from 40 to 140% in these experiments. Thus preferential synthesis may occur under some conditions, but it is small in magnitude and not a consistent finding.

Rates of acetylase synthesis are also presented in Table I. Although

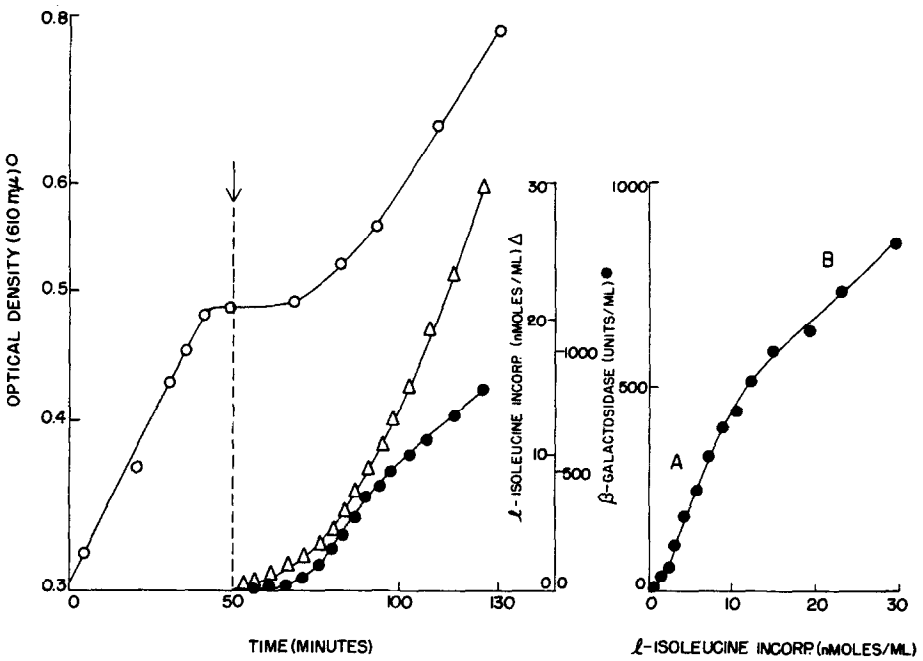

Figure 1. Left: strain 200PS/Flac was grown in medium 63 (Cohen and Rickenberg, 1956) supplemented with thiamine 2 ug/ml, glucose 400 ug/ml and lactose 2 mg/ml. At the arrow C^{14} l-isoleucine (0.02 uC and 5 x 10^{-8} moles/ml) was added. l-isoleucine incorporation was measured by collecting bacteria on a membrane filter (Millipore), washing with cold 5% trichloracetic acid and counting in a flow counter. β-galactosidase was measured by the rate of orthonitrophenyl-β-D-galactoside hydrolysis in toluene-treated cell suspensions. One unit of β-galactosidase produces one millimicromole of product per minute at 28°.

Right: a differential plot of β-galactosidase synthesis in this experiment.

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the ratio of acetylase activity to β-galactosidase activity varies somewhat, this variation does not exceed 30%. This finding is in contrast to the conclusions drawn earlier (Naono et al., 1965) on the basis of fractionation of proteins labeled during diauxie. Three peaks of radioactivity of approximately equal size were found, and since one of the peaks coincided with the peak of acetylase activity, this result could be taken to imply that acetylase represents a major fraction of all proteins synthesized during diauxie. But, since Zabin (1963) has shown that acetylase represents approximately 0.2% of all proteins in growing cells, it is more likely that the peak seen in the previous study was due to a fortuitous coincidence.

TABLE I

RATES OF ENZYME SYNTHESIS

Growth Conditions	β-galactosidase	Acetylase	Acetylase / β-galactosidase
	units per 10^{-9} moles of l-isoleucine incorporated		
Diauxie - A (early)	54	0.5	0.009
Diauxie - B (late)	18	0.14	0.008
Glycerol 1% IPTG 3×10^{-4} M	66	0.66	0.01
Lactose 0.5%	11	0.08	0.007

Acetylase was measured by a modification of the colorimetric procedure of Alpers et al., (1965). One unit of acetylase produces one millimicromole of product per minute at 37°.

Early and late diauxie refer to the periods indicated by the letters A and B respectively in Figure 1.

The changes in differential rate of synthesis during diauxie can be explained in the following way. After glucose exhaustion catabolite repression disappears and metabolism of lactose begins. Sufficient amounts of allo-lactose or other inducing compounds (Burstein et al., 1965) are gradually made, resulting in an increasing rate of synthesis of the products of the lactose operon. Early in diauxie, when but little induction has occurred, entry limits the utilization of lactose and so catabolite repression does not occur. Under these conditions β-galactosidase and acetylase synthesis proceed at approximately 80% of the rate in glycerol-grown IPTG-induced cells. Those experiments in which the rate early in diauxie exceeded that in glycerol-IPTG may represent ones in which catabolite repression early in diauxie was even lower than during growth on glycerol. Later, when entry of lactose no longer limits its utilization, catabolite repression appears and the rates of synthesis drop to levels close to those found in cells growing on lactose for many generations. Thus, although there is a period during diauxie when the differential rate of synthesis of two of the enzymes of the lactose

operon is rather high, the absolute rates of synthesis during diauxie are not higher than can be explained in terms of inducer and catabolite repression effects. This result does not impugn the earlier conclusion (Naono et al., 1965) that easily detectable amounts of lac-specific messenger RNA are synthesized during diauxie. It merely indicates that one should find approximately as much lac-specific messenger RNA early in diauxie as in fully induced growing cells, or at best 40% more under favorable conditions.

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