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DISCLAIMER

Intermediary Metabolite Levels in Strains of Escherichia coli Sensitive and Resistant to Catabolite Repression

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1. A number of strains of Escherichia coli were isolated which were capable of synthesizing β-galactosidase when induced by lactose in the presence either of glucose, or of glucose plus glucose 6-phosphate plus gluconate plus casein hydrolysate. 2. In no case was the reduced susceptibility to catabolite repression genetically linked to the lactose operon. 3. In all of the resistant strains, the sensitivity to catabolite repression of tryptophanase was also reduced. 4. Two resistant strains were investigated in more detail and compared with the parent organism. Resistance to catabolite repression for both β-galactosidase and tryptophanase was apparent only when glucose was the repressing substrate; catabolite repression exerted by other substrates was unchanged in the mutant strains. This was true even for the mutant isolated on the basis of inducibility by lactose in the combined presence of glucose, glucose 6-phosphate, gluconate and casein hydrolysate. However, both mutant strains were able to use glucose as the sole carbon source. 5. By growing the two mutants and the parent in media containing $^{32}P_i$, the concn. of 22 intermediary metabolites as a

function of the carbon source were determined by paper chromatography. The measurements referred to the concn. of the metabolites in the culture as a whole; separate determinations of metabolite concn. within the cells and in the external medium were not attempted. No correlation was found between metabolite concn. and severity of catabolite repression. 6. Enzymic determinations of the concn. of $NADP^+$ and NADPH in one mutant and the parent also failed to show a correlation with the rate of β-galactosidase synthesis. 7. In another series of strains no relation was found between repression of β-galactosidase synthesis and intracellular concn. of fructose 1:6-diphosphate or triose phosphate.

Several aspects of the mechanism of catabolite repression have not been satisfactorily elucidated. Silverstone, Magasanik, Reznikoff, Miller & Beckwith (1969) proposed the promoter, and hence the initiation of mRNA synthesis, as being the genetic site determing sensitivity of the lac enzymes to catabolite repression, although the details of such a mechanism remain obscure (Zubay, Schwartz & Beckwith, 1970). On the other hand, Moses & Yudkin (1968), Yudkin & Moses (1969) and Yudkin (1969a) have invoked regulation of the translational process as being a factor in the overall manifestation of the catabolite repression response. Some support for the latter view has been provided by Moses & Sharp (1970b).

Control of the translation stage in protein synthesis by catabolite repression might be expected to depend on a genetic determinant(s) in the structural genes of the operon. While Yudkin (1969b)

showed that no single locus could be responsible for coordinate catabolite repression affecting the translation of all the lactose enzymes, he suggested that repression of the various enzymes is separately effected, with equal degrees of effectiveness. We have therefore made a search for mutants in which the sensitivity of β-galactosidase synthesis to catabolite repression might be reduced by virtue of a mutation within the structural gene for that enzyme.

A second area of uncertainty refers to the chemical identity of the catabolite repression effector. Many substances have at one time or another been cast in this role (see review by Paigen & Williams, 1970), but doubt has always remained. Recently it has been proposed that catabolite repression results not from an effector acting positively to promote repression, but rather from the absence of a factor (perhaps 3':5'-cyclic-AMP) which is essential for enzyme synthesis (de Crombrugghe, Perlman, Varmus & Pastan, 1969; Zubay et al., 1970). However, Moses & Sharp (1970a) showed that in growing cells 3':5'-cyclic-AMP caused significant changes in the levels of several intermediary metabolites.

In the course of our experiments we have isolated a number of mutant strains whose sensitivity to catabolite repression was modified pleiotropically. It seemed probable that such modifications originated in mutational changes in aspects of intermediary metabolism. We have used two such mutant strains, and the parent from which they came, to measure intermediary metabolite concentrations in cultures growing in different media which permit different levels of induced β-galactosidase synthesis.

EXPERIMENTAL

Bacterial strains. Strain XA7010 ($F^- i^{del} p^{del} o^{del} z^{del}_{M41} y^+ Sm^R$) was obtained from Dr. J. R. Beckwith. Strain VP19 was constructed by inserting the episome $F' pro^+_{A,B} lac^+$ into strain XA7010, using selection on lactose-minimal agar. Met^- derivatives of XA7010 and VP19 were designated VP13 and VP18, respectively. Strains 3000 ($i^+ o^+ z^+ y^+$), LA12G ($i^+ o^+ z^+ y^+ CR^-$), z_{177}/z_4 ($i^-_3 o^+ z^-_{177} y^+ arg^- ade^-/F' i^- z^-_4 y^+$), and B were from Dr. E. Steers, Dr. W. F. Loomis, jun., Dr. C. Willson and Dr. M. R. Lunt, respectively.

Growth conditions and media. The standard minimal medium was M63 (Pardee & Prestidge, 1961) supplemented with methionine (50 μg/ml) where necessary, and with the appropriate carbon source at a concn. of 0.8 mg C/ml. For experiments using $^{32}P_i$, a low-phosphate minimal medium (LP medium) was employed (Prevost & Moses, 1967) containing carbon sources and methionine as desired. Cells in liquid culture were grown as described by Moses & Prevost (1966).

Enzyme induction and assays. These were performed by previously established methods: β-galactosidase (Palmer & Moses, 1968); D-serine deaminase and L-tryptophanase (Pardee & Prestidge, 1961). One enzyme unit is defined as that quantity giving rise to 1 nmole of product/min at 37°. In some comparative experiments we report enzyme activities in arbitrary units.

Intermediary metabolite concentrations. (a) Sugar phosphates and nucleotides. Cells were grown through several subcultures in LP medium containing the desired carbon source. Cells from an overnight culture were diluted into fresh LP medium containing $^{32}P_i$ (0.625 mCi/μmole) to give a concn. of about 11 μg bacterial protein/ml.

Samples (2 ml) were vigorously shaken at 37° in air in stoppered tubes (15 x 125 mm), held at an angle to promote maximum agitation. Growth was followed at 650 nm with a Spectronic 20 spectrophotometer (Bausch & Lomb, Rochester, N. Y., U.S.A.) masked down to permit reading 2 ml vol. of liquid in the tubes. When the cell density reached 115 μg bacterial protein/ml, 1 ml samples of the suspension were mixed with 4 ml ethanol. Portions (0.1 ml) of the ethanolic cell suspensions were chromatographed *in toto* on sheets of Ederol no. 202 paper (J. C. Binzer, G.m.b.H., Hatzfeld/Eder, Germany), using the solvent system described by Crowley, Moses & Ullrich (1963). Chromatograms were over-run to permit maximum separation of phosphorylated compounds. The labelled components were located on the chromatograms by radioautography. The spots were excised and the radioactivity measured automatically at known counting efficiency (Moses & Lonberg-Holm, 1963). The concn. of phosphorylated compounds were then calculated in terms of μmoles P/g bacterial protein. For spot identifications, authentic marker compounds were chromatographed on parallel chromatograms with the same labelled cell extract.

(b) $NADP^+$ and NADPH. Cells grown as in the previous paragraph (but in M63 medium) were sampled during exponential growth when the cell density reached 115 μg bacterial protein/ml. For subsequent measurements of $NADP^+$, 0.5 ml of cell suspension was mixed immediately with 0.1 ml N-HCl and heated at 60° for 10 min; for NADPH measurements, 0.6 ml of cell suspension was immediately mixed with 0.1 ml N-KOH and heated at 60° for 10 min. The concn. of $NADP^+$ and

NADPH in the solutions were measured by the cycling method of Lowry, Passoneau, Schultz & Rock (1961); standard calibration curves were constructed for each set of determinations.

(c) Fructose 1:6-diphosphate and triose phosphate. Cells in M63 medium containing the desired carbon source were grown exponentially to a measured density of 150-200 µg bacterial protein/ml. Samples (6 ml) of the suspension were mixed with 0.66 ml of 50% (w/v) trichloroacetic acid. Simultaneously, portions of the culture were filtered through a pad consisting (top to bottom) of one Whatman glass fibre disc GF/B, one Whatman glass fibre disc GF/C, and one Millipore filter disc (0.45 µ pore size). The Millipore filter ensured removal of the cells, but rapidly clogged unless the glass fibre discs were present as pre-filters. Portions of the filtrate (6 ml) were mixed with 0.66 ml of 50% (w/v) trichloroacetic acid. After 1 hr at room temp. (and after centrifugation for the whole suspension samples), the clear solutions were extracted 5 times with ether to remove trichloroacetic acid, and residual ether removed with a stream of air. The concn. of fructose 1:6-diphosphate and triose phosphate in the solutions were measured by the method of Bücher & Hohorst (1965). The concn. of the metabolites within the cells was calculated from the difference in concn. between the whole suspension and the filtered medium.

Chemicals and radiochemicals. Carrier-free $^{32}P_i$ was obtained from International Chemical and Nuclear Corp., Irvine, Calif., U.S.A. Fructose 1:6-diphosphate, N-acetylglucosamine, NADPH and α-oxoglutarate were from Calbiochem, Los Angeles, Calif., U.S.A.; dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and $NADP^+$ were from Sigma

Chemical Co., St. Louis, Mo., U.S.A.; IPTG* and BCIG were from Cyclo Chemical Corp., Los Angeles, Calif., U.S.A.; NTG was from Aldrich Chemical Co., Gardena, Calif., U.S.A.; EMS was from K & K Laboratories Inc., Plainview, N. Y., U.S.A.; spectinomycin sulphate was a gift from Dr. G. B. Whitfield, jun., The Upjohn Co., Kalamazoo, Mich., U.S.A.; N-acetyllactosamine (4-O-β-D-galactopyranosyl-N-acetyl-D-glucosamine) was a gift from Dr. R. M. Tomarelli, Wyeth Laboratories Inc., Philadelphia, Pa., U.S.A.

Enzymes for the assays of $NADP^+$, NADPH, fructose 1:6-diphosphate and triose phosphate were purchased as follows: rabbit muscle ketose 1-phosphate aldehyde lyase (EC 4.1.2.7), yeast D-glyceraldehyde 3-phosphate: NAD oxidoreductase (EC 1.2.1.12), rabbit muscle D-glyceraldehyde 3-phosphate ketol-isomerase (EC 5.3.1.1), beef liver L-glutamate:NAD oxidoreductase (EC 1.4.1.2) and yeast 6-phospho-D-gluconate:NADP oxidoreductase (EC 1.1.1.44) were from Boehringer Mannheim Corp., San Francisco, Calif., U.S.A. Yeast D-glucose 6-phosphate:NADP oxidoreductase (EC 1.1.1.49) was purchased from Calbiochem, Los Angeles, Calif., U.S.A.

RESULTS

Isolation and properties of strains resistant to catabolite repression. Loomis & Magasanik (1965) obtained catabolite repression-resistant mutants (e.g. LA12G) by supplying N-acetyllactosamine as the sole

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*Abbreviations: IPTG, isopropyl-thio-β-D-galactoside; BCIG, 5-bromo-4-chloro-3-indolyl-β-D-galactoside; NTG, N-methyl-N'-nitro-N-nitrosoguanidine; EMS, ethyl methane sulfonate.

source of N in the presence of large amounts of glucose. This compound requires prior hydrolysis to N-acetylglucosamine by cellular β-galactosidase before the N becomes available for growth. We have not found this to be a satisfactory way of obtaining resistant mutants. The material itself is difficult to synthesize, and small amounts only were available to us through the generosity of Dr. Tomarelli. Tests of the method with known resistant (LA12G) and sensitive strains showed that it is difficult to obtain clear-cut distinctions in the small vol. cultures necessitated by the limited availability of the N-source.

Another method of obtaining catabolite repression-resistant mutants was then employed using strains VP18 and VP19. These strains carry F'$\underline{lac}^+$ episomes in strain XA7010, and it was important to know the catabolite repression behaviour of the lactose enzymes in the latter strain. β-Galactosidase is partially deleted (Beckwith, 1964), and we have been unable to detect thio-galactoside transacetylase activity in this strain. Since the accurate measurement of galactoside permease activity presents a number of complications, we have studied β-galactosidase synthesis in partial diploids in which the enzyme is formed by *in vivo* complementation.

The M41 deletion in XA7010 is known to terminate within the α-cistron of the *z* gene (Beckwith, 1964). The point mutant $\underline{z}_4^-$ lies in the β-cistron (Ullmann, Jacob & Monod, 1967), and episomes carrying this mutation complement well with chromosomes carrying $\underline{z}_{177}^-$, a point mutation in the ω-cistron. The episome F' $\underline{i_3^- z_4^- y^+}$ was inserted from strain z_{177}/z_4 into XA7010, and F-ductants isolated on lactose-minimal plates lacking arginine and adenine. Catabolite repression

responses of strains z_{177}/z_4, XA7010/F' $\underline{i^-z_4^-y^+}$ and VP 19 (i.e. XA7010/F' $\underline{pro^+_{A,B}lac^+}$) are all typical (Table 1). As expected, XA7010 complements well *in vivo* with F' $\underline{i^-z_4^-y^+}$.

[INSERT TABLE 1 NEAR HERE]

Resistant mutants were isolated by mutagenizing cultures of VP19 with NTG (Adelberg, Mandel & Chen, 1965), EMS (Lin, Lerner & Jorgensen, 1962) or HNO_2 (Kaudewitz, 1959). Mutagenized cultures were spread on agar plates (1-1.5 x 10^4 cells/plate) of minimal medium (Loomis & Magasanik, 1967) containing 1% (w/v) glucose, 0.2% lactose (Loomis & Magasanik, 1965) and BCIG (40 μg/ml) (Davies & Jacob, 1968). The most suitable incubation schedule was 24 hr at 37° followed by 24 hr at room temp. Colonies containing β-galactosidase under these conditions appeared in varying ghades of blue. No blue colonies were found among approx. 75,000 colonies of unmutagenized VP19. A total of 234 blue colonies were picked and purified by restreaking on the same medium; 3 were from mutagenesis with HNO_2 (which produced a very low yield), 120 from NTG and 111 from EMS. Preliminary tests showed that 5 isolates were constitutive for β-galactosidase (2 from NTG and 3 from EMS), and 25 grew poorly or not at all on glucose. At least two of the latter were defective in glucose uptake (Brewer & Moses, 197).

Most of the remaining 204 isolates were resistant to catabolite repression to varying degrees, but in each case resistance for β-galactosidase synthesis was accompanied by resistance for tryptophanase synthesis. Thus, no strains were isolated uniquely resistant for the lactose enzymes, and this was confirmed genetically in many cases. A spectinomycin-resistant derivative of XA7010 was isolated

after treatment with NTG and selection on plates containing spectinomycin (135 μg/ml). The F'lac episomes from 61 of the catabolite repression-resistant strains were transferred into XA7010 Sp^R, and partial diploids isolated by selection on lactose-spectinomycin plates. In all cases the partial diploids were typically sensitive to catabolite repression. One of the strongly catabolite repression-resistant strains was designated VP21, and is discussed in more detail below.

Mutagenesis with NTG was repeated with strain VP18, the NTG-treated cultures then being spread on minimal agar plates containing BCIG as before, and supplemented with glucose (1% w/v) plus glucose 6-phosphate (0.5%) plus gluconate (1% w/v) plus casein hydrolysate (0.1%) plus lactose (0.2%) plus methionine (100 μg/ml). The intention was to avoid the appearance of mutants resistant by virtue of an alteration in the metabolism of one substrate only. Again, a variety of blue colonies were picked and purified. Of 35 tested, 6 were constitutive and sensitive to catabolite repression. Of the remaining 29 isolates, all were inducible but only 4 were clearly resistant. In those 4 cases resistance was observed for both β-galactosidase and tryptophanase synthesis; one of these strains (VP20) is considered in more detail below.

The F'lac episomes of the 4 strains were transferred into strain XA7010, with selection on lactose-minimal medium without methionine. All 4 F-ductants were sensitive to catabolite repression.

Enzyme synthesis in catabolite repression-resistant strains. Strains VP20 and VP21 were fairly resistant to catabolite repression by glucose, but remained sensitive to repression by other substrates. In

this regard they resemble strain LA12G (Moses & Yudkin, 1968; Rickenberg, Hsie & Janecek, 1968). When induced by 0.5 mM-IPTG, the relative differential rates of β-galactosidase synthesis in glycerol and glucose media were 100 and 29, respectively, for the parent VP19, and 100 and 91, respectively, for the mutant VP21. Induction by 6 mM-lactose yielded relative rates of 100 in glycerol and 0 in glucose for VP19, compared with 100 and 101 for VP21. In another experiment, VP19 and VP21 in glycerol and glucose media were simultaneously induced for β-galactosidase, tryptophanase and D-serine deaminase. The % of the rates of synthesis in glycerol which were found for the three enzymes in glucose were 40, 33 and 31, respectively, for VP19, and 130, 182 and 174, respectively, for VP21. Resistance to glucose in VP21 thus applied to at least two other enzymes in addition to β-galactosidase, although the isolation procedure was based upon resistance for the last of these only. Strain VP21 was very sensitive to repression by glucose and glycerol when deprived of a source of N (Table 2). Transient repression

[INSERT TABLE 2 NEAR HERE]

(Moses & Prevost, 1966; Paigen, 1966) of β-galactosidase synthesis by glucose was less pronounced in VP21 than in VP19, but was nevertheless apparent (Fig. 1).

[INSERT FIG. 1 NEAR HERE]

A comparison of the relative differential rates of β-galactosidase and tryptophanase synthesis, and of the relative rates of growth, for strains VP19, VP20 and VP21 in M63 and LP media supplemented with various carbon sources is shown in Table 3. The main differences

[INSERT TABLE 3 NEAR HERE]

between the mutant strains and the parent are seen in the comparative resistance of the synthesis of both enzymes in the mutant strains to repression by glucose, glucose plus casein hydrolysate, and, to a lesser extent, by maltose. Resistance to other substrates remained more or less unchanged. We conclude that the lesion in VP20 and VP21 affects primarily an aspect of glucose metabolism.

Intermediary metabolite concentrations. The concn. of 17 identified and 5 unifentified phosphorylated intermediates in VP19, VP20 and VP21 growing exponentially in LP medium with 5 carbon sources are shown in Table 4. The concn. of $NADP^+$ and NADPH in VP19 and VP21

[INSERT TABLE 4 NEAR HERE]

cultures in M63 medium with the same 5 carbon sources are presented in Table 5. Finally, the intracellular concn. of fructose 1:6-

[INSERT TABLE 5 NEAR HERE]

diphosphate and triose phosphate in strains B, LA12G and 3000 are shown for exponential and N-starved cells in Table 6 together with comparative data for induced β-galactosidase synthesis.

[INSERT TABLE 6 NEAR HERE]

DISCUSSION

The search for mutants resistant to catabolite repression. The results of Moses & Yudkin (1968), Yudkin & Moses (1969) and Yudkin (1969a) suggest that part of the control mechanism for catabolite repression resides in the translation of *lac* mRNA into enzyme protein. A plausible explanation for such a mechanism is that the nascent enzyme protein interacts specifically with the repression effector in such a way as to cause conformational changes in the former which

result in impairment or cessation of translation (Vogel, 1957; Szilard, 1960). The requirement for specificity implies that translation of nascent protein must proceed far enough for the development of sufficient secondary and tertiary structure to allow the precise recognition of protein and effector. This, in turn, would suggest the likelihood, but not the certainty, that if change in a single genetic locus could confer resistance to catabolte repression by destroying nascent protein-effector interaction, such a site would be translated relatively late during protein synthesis, and would lie in the distal portion of the structural gene.

With these considerations in mind, strains VP18 and VP19 were chosen as candidates from which to attempt to produce resistant mutants. The chromosome of these strains is deleted from an unknown point proximal to the i gene to a point within the α-cistron of β-galactosidase. The i, p and o genes are all deleted, and galactoside permease is synthesized constitutively at 25% of the wild-type rate (Beckwith, 1964). However, the β-cistron of β-galactosidase must be synthesized constitutively at the full wild-type rate, as shown by in vivo complementation (Table 1).

Catabolite repression, both in wild-type strains induced with high concn. of IPTG, and in constitutives, typically reduces the rate of β-galactosidase synthesis in glucose medium to about 30% of that in glycerol medium. This was also the case for β-galactosidase synthesis in partial diploids of XA7010. We recognized that if a control point exists in the distal part of the β-galactosidase structural gene, mutation to resistance in the episomal z gene of

VP18 and VP19 might be accompanied by simultaneous destruction of catalytic activity in the completed enzyme molecule. By employing partial diploids of XA7010, we expected that even should this occur, the synthesis of appreciable quantities of the β- and ω-cistrons on the chromosomal z gene, even under conditions of catabolite repression, would restore a measure of catalytic activity by in vivo complementation, and permit recognition of the mutant on the discrimination medium.

Such mutants were not found among more than 250 candidates examined. Inevitably, negative findings in this type of search do not permit unequivocal conclusions to be drawn. A number of possible explanations present themselves. The original hypothesis might be incorrect, and no single mutation might be capable of producing resistance to repression in the translational process. Alternatively, such mutations might be very rare, and the search for them not sufficiently extensive. Or, even if the theory is correct, and a single site does exist, it might conceivably lie in the α-cistron. If the altered α-cistron failed to produce a catalytically active enzyme, it would not have been detected in the present work since no provision was made to supply an alternative α-cistron for complementation. Our studies thus leave undecided the location of a genetic site controlling translational repression.

Intermediary metabolism and catabolite repression. Many substances have been proposed as the catabolite repression effector, including direct products of carbohydrate metabolism and such coenzymes as ATP and nicotinamide adenine dinucleotides. Some have been apparently

excluded by direct biochemical investigation or by indirect genetic methods. Loomis & Magasanik (1966) suggested, on the basis of studies with mutants blocked at known enzymes, that if a single compound is responsible it is related to the pentoses and trioses of intermediary metabolism. Moses & Sharp (1970a,b), following an earlier suggestion by Prevost & Moses (1967), have favoured a relation with the pentose phosphate pathway or one of its products. Dobrogosz (1968a,b) suggested that the effector is N-acetylglucosamine 6-phosphate. Tyler, Wishnow, Loomis & Magasanik (1969) eliminated glucose 6-phosphate, while Hsie, Rickenberg, Schultz & Kirsch (1969) have additionally removed 6-phosphogluconate and NADPH from the list of candidates. Our present data confirm these results, and we have extended the list to include a further 17 identified and 5 unidentified metabolites. None of our analyses show a correlation between the gross amount in the culture of any metabolite investigated and the severity of catabolite repression.

A recent alternative suggestion is that β-galactosidase synthesis is dependent upon intracellular 3':5'-cyclic AMP (de Crombrugghe et al., 1969), and that the concn. of these substances inside the cells is reduced in conditions giving rise to catabolite repression (Makman & Sutherland, 1965; H. E. Varmus, R. Perlman & I. Pastan, unpublished work). Our data (Table 4) show no correlation between total 3':5'-cyclic AMP in the system and catabolite repression, but we have not separately measured the intra- and extra-cellular concn. of this substance.

None of these studies have faced the problem of intracellular compartmentalization, in which measurement of the gross concn. of

metabolites in the cells, or in the culture, is no reliable guide to their concn. at the target site (Prevost & Moses, 1967). The use of cell-free systems might overcome this difficulty, and the recent finding by Zubay et al. (1970), that 3':5'-cyclic AMP may be a necessary factor in β-galactosidase synthesis, is the strongest support to date for any one of the many substances proposed as an effector. While the intracellular concn. of 3':5'-cyclic AMP may indeed turn out to be the decisive factor in catabolite repression, the way in which this parameter responds to the varying physiological state of the cells is not presently understood. We find it surprising that cells grown on different carbon sources, resulting in widely differing rates of growth and of enzyme synthesis, should show such constant overall pool sizes for many metabolites. Even an immediate product of one of the growth substrates often showed comparatively little change when a different growth substrate was substituted. On the other hand, the protein composition of cells in a variety of media do exhibit a number of pronounced differences (Moses & Wild, 1969; Moses & Sharp, 197). We suspect that the explanation for many of these interrelated phenomena will come only when more is understood of the details of intracellular organization.

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CAPTION FOR FIGURE

Fig. 1. Transient repression in strains VP19 and VP21. Cells growing in glycerol-minimal medium were induced with 0.5 mM-IPTG. Glucose (10 mM) was added as shown by the arrows. Mass doubling times before and after adding glucose: VP19, 84 min and 64 min, respectively; VP21, 84 min and 60 min, respectively. o, VP19; o, VP21.

Running title: Intermediary metabolite levels in E. coli

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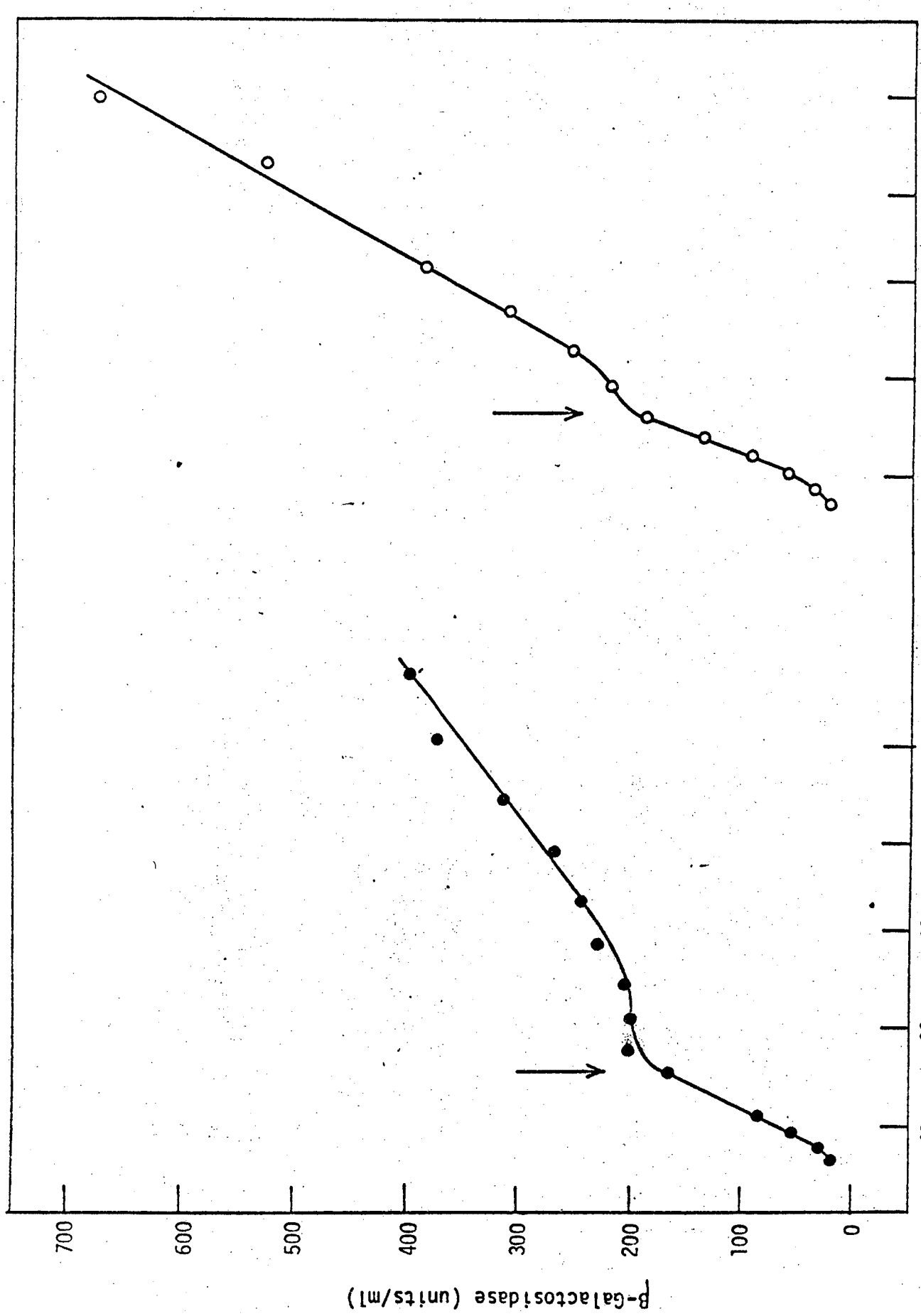
β-Galactosidase (units/ml)
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Bacterial protein (μg/ml)
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60
80
100
120

Fig. 1

Table 1. Catabolite repression responses of some partial diploids

The differential rates of β-galactosidase synthesis in three partial diploids are reported for cells growing exponentially in glycerol- and glucose-minimal medium.

Strain	Differential rates of β-galactosidase synthesis (enzyme units/mg of bacterial protein)	
	Glycerol medium	Glucose medium
z_{177}/z_4	16,400	5,690
XA7010/F' $\underline{i^-_3 z^-_4 y^+}$	22,600	2,980
VP19*	17,900	5,560

*Induced with 0.5 mM-IPTG.

Table 2. β-Galactosidase synthesis in VP19 and VP21 in the absence of a source of N

During exponential growth in glycerol-minimal medium, the cells were filtered, washed and resuspended in M63 medium plus glycerol without N. After 15 min at 37° the cells were again harvested, washed, resuspended in M63 medium devoid of both glycerol and N, and incubated for a further 30 min at 37°. IPTG (0.5 mM) was then added, the cultures divided into five equal portions and supplemented as shown below. The enzyme activity synthesized in the first 30 min of induction is reported.

Supplements	β-Galactosidase activity (relative values)	
	VP19	VP21
None	100	100
Glycerol (22 mM)	3	1
Glucose (10 mM)	3	5
$(NH_4)_2SO_4$ (15 mM)	990	610
Glycerol (22 mM) + $(NH_4)_2SO_4$ (15 mM)	4400	3000

Table 3. Growth and enzyme synthesis in the parent strain VP19 and two mutants VP20 and VP21

The cells were simultaneously induced with 0.5 mM-IPTG and 2.5 mM-tryptophan during growth in the media listed. The values reported are relative to those obtained with ribose.

	M63 medium									Low P_i medium								
	VP19			VP20			VP21			VP19			VP20			VP21		
Carbon source	β-galactosidase	tryptophanase	growth rate	β-galactosidase	tryptophanase	growth rate	β-galactosidase	tryptophanase	growth rate	β-galactosidase	tryptophanase	growth rate	β-galactosidase	tryptophanase	growth rate	β-galactosidase	tryptophanase	growth rate
Acetate	98	80	102	-	-	-	84	101	78	125	238	41	100	331	59	91	224	63
N-Acetylglucosamine	56	16	159	52	16	124	37	15	127	-	-	-	-	-	-	-	-	-
Fructose	89	48	126	61	42	125	81	47	108	-	-	-	-	-	-	-	-	-
Galactose	43	42	147	16	67	81	52	56	123	-	-	-	-	-	-	-	-	-
Gluconate	51	15	128	22	0	159	30	10	109	34	5	148	29	5	151	29	4	224
Glucose	32	5	162	76	102	94	80	77	88	35	6	148	84	86	98	117	71	141
Glucose 6-phosphate	13	3	188	21	2	138	18	6	122	-	-	-	-	-	-	-	-	-
Glucose + tryptone	8	2	279	-	-	-	48	13	275	-	-	-	-	-	-	-	-	-
Glycerol	99	43	116	89	47	112	73	37	88	83	59	121	92	76	104	65	35	174
Lactate	98	81	102	-	-	-	102	99	86	-	-	-	-	-	-	-	-	-
Lactose	44	12	140	29	13	124	42	18	134	-	-	-	-	-	-	-	-	-
Maltose	59	56	140	70	49	103	73	70	95	-	-	-	-	-	-	-	-	-
Ribose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Succinate	87	56	124	-	-	-	103	73	108	-	-	-	-	-	-	-	-	-

Table 4. Concn. of phosphorylated metabolites in parent strain and two mutants

Cells were grown exponentially in LP medium containing $^{32}P_i$ and one of five carbon sources. The analytical procedure is described in the Experimental section. Concn. are expressed in terms of μmoles P/g bacterial protein. The growth rates and differential rates of β-galactosidase synthesis for these strains under similar conditions are presented in Table 3.

carbon source.....	acetate			gluconate			glucose			glycerol			ribose		
bacterial strain....	VP19	VP20	VP21	VP19	VP20	VP21	VP19	VP20	VP21	VP19	VP20	VP21	VP19	VP20	VP21
Metabolite															
ADP*	23.3	17.0	21.5	13.8	14.7	16.5	14.2	14.2	17.2	10.8	13.9	17.0	13.0	13.1	14.3
AMP*	-	2.1	4.8	-	3.4	5.3	3.0	6.1	4.7	2.5	-	2.6	2.1	2.8	3.7
ATP*	8.4	13.9	15.0	16.0	15.9	19.5	15.7	12.9	20.0	13.6	12.4	30.6	11.0	8.4	12.6
CMP*	6.4	3.4	2.5	6.1	8.0	12.5	4.1	11.4	13.7	4.9	7.5		5.0	5.8	9.0
3':5'-Cyclic AMP*	3.6	8.8	4.5	5.1	6.1	5.8	6.0	9.1	9.0	5.7	7.5	6.7	5.0	6.1	5.6
Dihydroxyacetone phosphate*	8.2	8.7	4.8	4.7	8.0	5.3	3.8	8.0	5.6	5.9	7.2	5.7	5.1	6.4	4.9
Disaccharide phosphate**	12.5	17.0	17.8	15.7	17.3	20.7	15.7	19.0	20.1	16.4	17.3	14.3	14.5	16.0	15.9
Fructose 6-phosphate*	9.0	14.0	9.9	18.2	32.7	33.1	13.1	26.0	18.7	10.8	23.0	14.8	15.4	21.1	14.1
Fructose 1:6-diphosphate*ø	20.1	22.4	26.7	41.7	87.3	85.8	55.3	73.7	63.1	45.4	50.2	59.7	47.9	54.3	56.0
UTP*ø	4.6	6.3	6.3	33.2											
Glucose 6-phosphate*	12.6	8.4	8.3	12.2	18.1	14.2	12.3	17.8	19.3	7.5	12.6	9.8	16.8	18.6	14.1
Phosphoenolpyruvic acid*	3.2	5.9	4.3	1.0	3.9	2.3	0.8	3.2	1.9	3.0	6.0	2.9	1.4	2.3	1.4
3-Phosphoglyceric acid*	6.2	10.8	7.4	9.2	28.6	16.5	6.6	13.9	9.9	11.8	23.6	14.6	7.7	13.4	6.8
Phosphoglycollic acid**	0	0	0	2.0	2.4	2.7	2.2	2.6	32.7	3.3	2.6	4.1	6.7	2.3	13.6
6-Phosphogluconic acid*	0.4	0.5	0.7	7.8	13.9	13.6	1.6	1.4	1.8	0.8	1.2	1.3	1.0	1.5	1.1
Ribose 5-phosphate*	4.9	4.7	7.7	7.7	8.1	8.6	8.5	11.5	9.7	16.2	8.0	9.2	6.1	8.0	7.7
UDP-glucose*	3.7	4.7	4.5	4.6	5.8	7.0	4.3	5.1	7.0	3.4	4.1	5.1	2.8	4.0	3.8
Unidentified 1	0	0	0	2.0	2.7	3.1	0	0	0	0	0	0	0	0	0
Unidentified 2	4.6	9.5	4.2	5.1	8.1	8.4	5.9	11.2	8.2	7.0	7.7	7.5	6.1	6.8	6.7
Unidentified 3	4.1	5.2	5.2	3.8	4.4	7.1	3.7	6.0	6.6	5.8	6.1	6.1	3.6	7.2	5.0
Unidentified 4	6.4	7.5	7.5	-	-	5.6	6.3	7.9	6.7	5.7	6.5	7.3	6.5	-	8.4
Unidentified 5	-	4.6	2.1	-	5.8	-	0.9	1.6	-	1.7	-	1.5	2.1	4.2	-

* identity checked by cochromatography with authentic marker in this work
** identity inferred from other chromatographic studies
ø separation often incomplete

Table 5. Concn. of $NADP^+$ and NADPH in cultures of VP19 and VP21 in five different media

Cells were sampled during exponential growth. Sampling and assay procedures are described in the Experimental section. Results are reported in terms of μmoles P/g bacterial protein, corresponding with the units used in Table 4.

	VP19		VP21	
Carbon source	$NADP^+$	NADPH	$NADP^+$	NADPH
Acetate	7.1	12.6	4.6	22.4
Gluconate	6.2	12.8	9.1	21.0
Glucose	6.3	13.9	12.6	23.4
Glycerol	4.6	11.5	9.1	24.0
Ribose	6.4	16.5	7.9	23.1

Table 6. Concentrations of fructose 1:6-diphosphate and of triose phosphate in cells of three strains of E. coli in different physiological conditions

Concn. expressed as μmoles/g bacterial protein. Differential rates of β-galactosidase synthesis expressed as relative values in upper section; lower section compares absolute rates of enzyme synthesis during 30 min period after adding 0.5 mM-IPTG to equal amounts of cell. Nitrogen-starved cells were prepared as described in the caption to Table 2.

Strain.....	3000			B			LA12G		
Exponential growth	FDP	TP	Enzyme Synthesis	FDP	TP	Enzyme Synthesis	FDP	TP	Enzyme Synthesis
Carbon source:									
Glycerol	15.1	15.4	100	15.3	15.0	100	9.4	12.0	100
Glycerol + CA	17.2	19.4	50	14.3	7.2	73	6.7	9.3	55
Glucose	12.8	5.5	52	25.2	10.8	36	12.3	4.7	63
Glucose + CA	12.5	2.0	35	16.7	5.0	23	7.9	5.0	55
Succinate	0.54	1.7	100	-	-	-	-	-	-
Lactate	-	-	-	0.23	0.0	100	0.0	0.0	100
In absence of nitrogen									
Additions:									
None	0.30	2.5	100	1.4	5.4	100	1.4	0.0	100
Glycerol	19.7	14.3	0	2.4	0.0	174	5.0	0.0	0
Glucose	11.3	8.3	0	11.9	10.0	7	17.1	8.6	4
Succinate*	3.7	5.9	0	-	-	-	-	-	-
Lactate**	-	-	-	0.0	2.0	22	0.0	1.9	4

* in cells previously grown on succinate
** in cells previously grown on lactate

FDP, fructose 1:6-diphosphate; TP, triose phosphate; CA, casein hydrolyzate.

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