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Growth-Rate-Dependent Alteration of 6-Phosphogluconate Dehydrogenase and Glucose 6-Phosphate Dehydrogenase Levels in *Escherichia coli* K-12

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The levels of 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase are subject to metabolic regulation; they increased three- to fivefold with increasing growth rate.

It is generally accepted that the levels of the enzymes of glycolysis and the hexose monophosphate shunt in enteric bacteria vary little, if at all, in cells growing under different nutrient conditions, even though experimental data supporting this assertion are scarce (3). Our interest in studying the mechanism of expression of *gnd* (5), the gene coding for 6-phosphogluconate dehydrogenase, led us to examine in *Escherichia coli* K-12 the effect of growth rate on the amount of this enzyme and of glucose 6-phosphate dehydrogenase.

For this study we chose strain W3110, a wild-type strain of *E. coli* K-12 (1) often used for genetic and physiological studies (10). Growth experiments were carried out using morpholinepropanesulfonic acid (MOPS) medium supplemented with various carbon sources and the methods for physiological studies described in detail by Neidhardt et al. (8) and Wanner et al. (13).

To study the effect of carbon source on specific activity, cells of strain W3110 were grown in media of differing nutritional compositions. The specific growth rate constant varied more than 10-fold (Fig. 1). The specific activity of 6-phosphogluconate dehydrogenase (Fig. 1A) increased four- to fivefold with increasing growth rate in the range of specific growth rates between 0.1 and 0.5 and was no higher in faster-growing cells. The specific activity of glucose 6-phosphate dehydrogenase varied with growth rate in a similar manner (Fig. 1B). Similar results for both enzymes were obtained when medium 63 (12) was substituted for MOPS medium (data not shown).

One possible explanation for the data shown in Fig. 1 is that the various sonic extracts contained inhibitors or stimulators of the activities of the respective enzymes which varied with growth rate. To test for such factors, we dialyzed exhaustively against sonication buffer sonic extracts prepared from cultures grown in glucose MOPS and acetate MOPS media. The specific activities of the extracts before and after dialysis were the same. Further, the activity of each enzyme was strictly additive when the extracts were mixed in several different proportions (data not shown).

Growth of *E. coli* on sugars transported by the phosphoenolpyruvate:sugar phosphotransferase system (PTS) leads to a threefold induction of enzyme I and HPr, the non-sugar-specific proteins of the PTS (11). Synthesis of 6-phosphogluconate dehydrogenase is not coupled to this regulatory unit because its specific activity is as high in cells grown on the non-PTS sugars, glycerol, lactose, maltose, and arabinose, as in cells grown on glucose which is transported by the PTS (Fig. 1A).

The variation in specific activities observed for the two enzymes could be due to growth rate per se or to media-specific effects which result from differences in the concentration of various metabolites or the actual metabolic pathways used during growth under the various conditions. To distinguish between these possibilities, we varied the specific growth rate while holding constant the carbon source. This was accomplished by the method of Hansen et al. (4), in which the specific growth rate of cells growing on glucose is decreased by increasing the ratio of α-methylglucose (α-MG) to glucose in the medium, α-MG being a non-metabolizable analog of glucose which limits growth by competing with glucose for transport. Figure 2 shows that incremental increases in the ratio of α-MG to glucose from 0 to 40 resulted in incremental decreases in the specific growth rate of strain W3110 from 0.58 to 0.21. Under these conditions the specific activities of both enzymes decreased about 2.0- to 2.5-fold with decreasing growth

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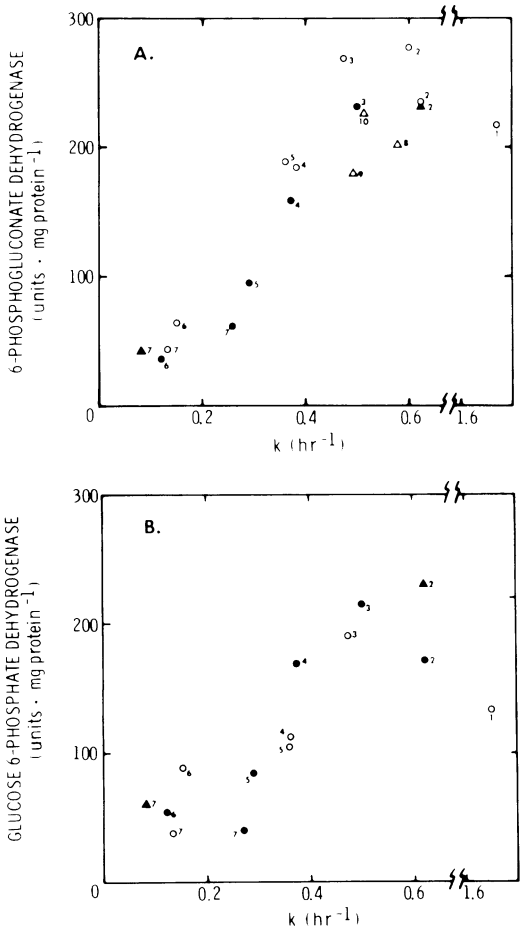

FIG. 1. *Effect of carbon source on the specific activity of (A) 6-phosphogluconate dehydrogenase and (B) glucose 6-phosphate dehydrogenase. Strain W3110 was obtained from L. Soll; that it is E. coli K-12 was verified by its sensitivity to λcI. The inoculum for each growth experiment was obtained by diluting a frozen glycerol suspension to 10^5 cells per ml in MOPS medium containing 1.9 mM glucose or 1.9 mM pyruvate and incubating at 37°C; these conditions support exponential growth to an absorbance at 420 nm (A_{420}) of about 1.0, at which point the available carbon source is exhausted and growth ceases abruptly (8). (A culture of strain W3110 grown in glucose MOPS medium to an A_{420} of 1.0 contains 5.4×10^8 cells per ml, as determined by measurement of viable count.) Bacteria arrested in exponential growth phase were inoculated into the various media at densities between 10^4 and 10^5 cells per ml. The final concentrations of carbon sources and the composition of fully supplemented medium were those of Neidhardt et al. (8). Cultures were grown aerobically at 37°C in baffled-bottom flasks. Growth was monitored turbidimetrically by measuring A_{420} nm. Growth rates were determined at culture densities between an A_{420} of 0.05 and 1.0, with cultures above an A_{420} of 0.3 being suitably diluted before measurement of A_{420}. Growth rates are expressed in terms of the specific growth rate constant, k, calculated from the expression k = (ln2)/(mass doubling time [hours]). At an A_{420} of ca. 0.5, two samples were taken and added to prechilled tubes containing chloramphenicol at a final concentration of 100 μg/ml. Cells were collected by centrifugation, washed, suspended in 0.2 volume of sonication buffer and disrupted by 50 s of sonic treatment as described previously (15). Cell debris was removed by centrifugation. Activity of 6-phosphogluconate dehydrogenase and glucose 6-phosphate dehydrogenase in sonic supernatant fluids was assayed spectrophotometrically by measuring the rate of formation of NADPH in the assay system described for each enzyme by Fraenkel and Levisohn (2). One enzyme unit is equivalent to 1 nmol of NADPH formed per min at 25°C. Only truly linear initial velocities were used for activity determinations; they were obtained when the amount of sonic supernatant fluid assayed yielded a rate of formation of NADPH in the range of 0.5 to 5 units. Protein was measured by the method of Lowry et al. (7), using bovine serum albumin corrected for moisture content (6) as the standard. Standard deviations of measurements of specific activity were less than 10%. Control experiments using cells grown in glucose, serine, and acetate minimal medium showed that the specific activity of 6-phosphogluconate dehydrogenase was the same after four 50-s treatments as after one. The amount of protein released after one 50-s treatment was in each case greater than 85% of that released by four. Each point represents the average specific activity of the two samples taken from a single culture. Symbols (○, ●, △, ▲) represent independent experiments starting from different inocula. Numbers in the body of the figure indicate the following primary carbon sources and supplements to MOPS medium: (1) Glucose MOPS medium fully supplemented with 20 amino acids, four nucleotide bases, and five vitamins; (2) glucose; (3) gluconate; (4) glycerol; (5) pyruvate; (6) serine; (7) acetate; (8) lactose; (9) maltose; (10) arabinose.*

rate. Since varying growth rate by varying the ratio of α-MG to glucose had nearly the same effect, qualitatively and quantitatively, on the specific activities of the two enzymes as varying growth rate with different carbon sources (cf. Fig. 1 and 2), we conclude that the level of these enzymes depends on growth rate per se and not on media-specific effects.

An increase in specific activity with increasing growth rate could be due to an increase in enzyme concentration or to an increase in the activity of each enzyme molecule. To distinguish between these possibilities for 6-phosphogluconate dehydrogenase, we purified the enzyme and prepared antiserum directed against it by previously described methods (15). Figure 3 shows the neutralization of 6-phosphogluconate dehydrogenase activity in sonic extracts prepared from cells grown on glucose, serine, and acetate.

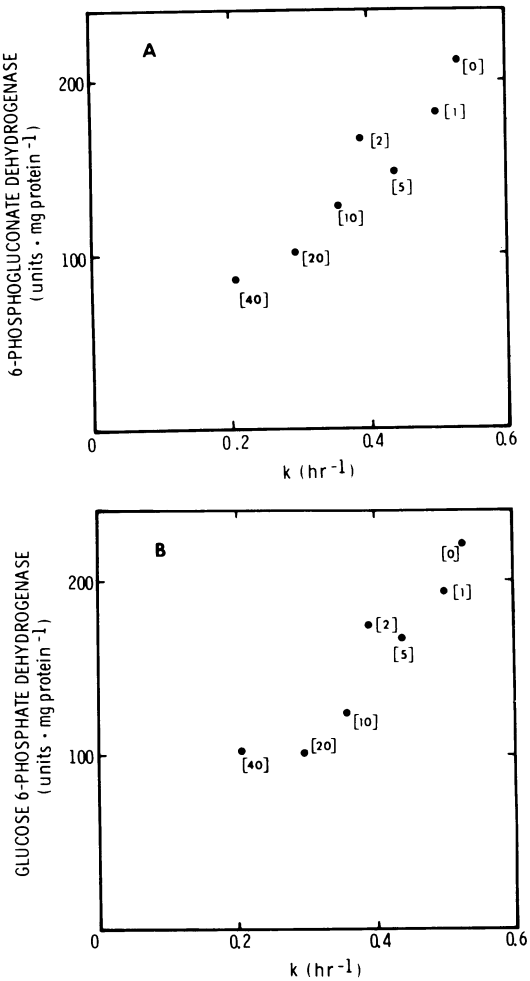

FIG. 2. *Specific activity of (A) 6-phosphogluconate dehydrogenase and (B) glucose-6-phosphate dehydrogenase in cultures of strain W3110 growing on glucose in the presence of increasing amounts of α-MG. MOPS medium was supplemented with 0.1% glucose (wt/vol) and α-MG to give the ratio shown in brackets. The inoculum was strain W3110 arrested in exponential phase of growth on limiting pyruvate. Cultures were maintained in exponential growth in the respective media for at least eight generations before two samples were taken at an absorbance at 420 nm of 0.5 and assayed for enzyme activity and protein. Exponential growth under these conditions continues to an absorbance at 420 nm greater than 1.0. All other conditions for growth and for measurement of specific activity were those given in the legend to Fig. 1.*

The amount of activity neutralized per unit of antiserum was the same in the three extracts. Thus, each unit of enzyme activity represents the same amount of enzyme protein, and changes in specific activity are due to changes in the amount of enzyme protein.

The results presented here show that in *E. coli* K-12 the amount of 6-phosphogluconate dehydrogenase varies with growth rate. This is not the case in *Salmonella typhimurium*. In *S. typhimurium* the specific activity is nearly invariant over the same range of growth rates examined here (14). Thus, related genera of bacteria may differ in the metabolic regulation of a given enzyme, perhaps reflecting an adaptation of the particular organism to its environment. In this regard it is interesting that (i) the specific

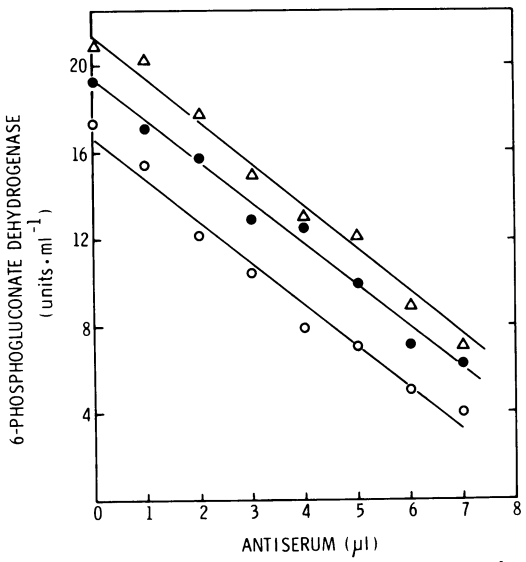

FIG. 3. *Neutralization of 6-phosphogluconate dehydrogenase activity by antiserum. Strain W3110 was grown in MOPS medium with glucose (k = 0.65), serine (k = 0.17), and acetate (k = 0.12) as carbon sources as described in the legend to Fig. 1. Sonic extracts were prepared and assayed for 6-phosphogluconate dehydrogenase activity and for protein as described in the legend to Fig. 1. The respective specific activity measurements were 205, 63, and 48 U per mg. The extracts from glucose- and serine-grown cells were diluted in sonication buffer containing bovine serum albumin (300 μg/ml) to give enzyme activities per milliliter about equal to that of the acetate-grown cells. 6-Phosphogluconate dehydrogenase was purified by affinity chromatography on blue dextran-Sepharose, and antiserum directed against it was prepared by methods described previously (15). Antiserum was diluted 1:100 in sodium phosphate buffer, pH 7.0, containing 0.15 M sodium chloride (phosphate-buffered saline). Increasing amounts of antiserum brought to 8 μl with phosphate-buffered saline were added to 100 μl of sonic extracts. Antigen-antibody complexes were allowed to form for 3 h at 4°C, after which time each sample was assayed for 6-phosphogluconate dehydrogenase activity. A 100-fold higher amount of preimmune serum had no effect on enzyme activity in sonic extracts. Symbols: ●, Glucose; △, serine; ○, acetate.*

activity of glucose 6-phosphate dehydrogenase in *E. coli* K-12 is about threefold higher in cells grown with glucose as the carbon source than with serine or acetate (Fig. 1B), whereas it is invariant when *E. coli* B/r is grown on the various carbon sources (13); (ii) *E. coli* K-12 grows at about half the rate on a given carbon source as *E. coli* B/r (comparing our data with that of Wanner et al. [13]). Thus, it is possible that the different growth properties of the two strains of *E. coli* stem from differences in the metabolic regulation of various enzymes.

Enzymes of glycolysis and the hexose monophosphate shunt are "constitutive," meaning that they are present under all growth conditions. Although the word constitutive is often mistakenly used to imply that synthesis of the respective enzymes is unregulated, the described dependence of enzyme level on growth rate, or for that matter an invariance in level, is more likely the result of an active regulatory process, as appears to be the case for the components of the protein-forming system and the subunits of RNA polymerase (9).

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