INDUCTION OF FRUCTOSE-1,6-DIPHOSPHATASE ACTIVITY IN ESCHERICHIA COLI

A.M. Gotto and Burton M. Pogell,* Departments of Microbiology and Biochemistry, Vanderbilt University School of Medicine, Nashville 5, Tennessee

Received October 8, 1962

The formation of carbohydrates from non-carbohydrate precursors (glyconeogenesis) is thought to require fructose-1,6-diphosphatase (FDPase) in order to bypass the highly exergonic phosphofructokinase reaction (see Krebs (1)). This is supported by the existence of a highly specific FDPase in liver which hydrolyzes the carbon-1 phosphate group of fructose-1,6-diphosphate (FDP) to form fructose-6-phosphate (2). Since in microorganisms the net formation of hexose for structural and functional components of the cell is necessary for growth on non-carbohydrate precursors, the effect of growth substrates on the level of FDPase was examined in Escherichia coli B**. The results showed the presence of high levels of FDPase during growth on acetate or alanine, intermediate levels during growth on lactate, ribose, or gluconate, and low levels during growth on glucose, fructose, or galactose (Table 1). It thus appeared that increased formation of FDPase occurred under conditions where a net formation of hexose from non-hexose precursors

* Research Career Development Awardee of the United States Public Health Service, No. GM-K3-3033.

** The carbohydrate content of E. coli has been reported to be 10 to 20% of its dry weight, the bulk of which is present as glycogen (3, 4).

was necessary. Increases in the level of acid p-nitrophenyl phosphatase under similar conditions have been reported previously by Hofsten (8).

TABLE 1

Specific Activity of Fructose-1,6-diphosphatase in Extracts of *Escherichia coli* B Grown on Different Substrates

Growth Substrate	Specific Activity (μmoles of inorganic phosphate formed/mgm. protein/hr.)
Acetate*	29.2, 30.5, 32.4, 35.3
L-Alanine	24.0, 28.8
D,L-Lactate	16.5, 18.9
D-Ribose	15.0, 15.5
D-Gluconate	13.2, 13.9
D-Galactose	5.7, 8.7
D-Fructose	2.7, 4.4
D-Glucose	3.2, 3.3, 1.2

*Addition of yeast extract to a concentration of 0.05% did not alter the level of FDPase.

Cells were grown with aeration at 37° on media which contained 50 mM growth substrate, 60 mM phosphate buffer (pH 7.0), 50 mM NH_4Cl, and salts (5). The cells were harvested by centrifugation, washed twice with 0.15 M KCl, resuspended in KCl, and disrupted by sonication. Debris was removed by centrifugation and FDPase and protein assayed in the supernatant solution. Protein was determined by the method of Lowry et al with bovine serum albumin as standard (6).

Enzyme assay: 2 μmoles of sodium FDP, 13.0 μmoles of sodium acetate (pH 4.7), and enzyme were incubated in a final volume of 0.2 ml. at 38°. The reaction was terminated by the addition of 0.2 ml. of 10% trichloroacetic acid, and inorganic phosphate determined in the supernatant by the method of Gomori (7).

Liberation of inorganic phosphate from FDP was linear with respect to enzyme concentration and time of incubation with both acetate- and glucose-grown extracts when no more than 30% of the substrate was hydrolyzed

The induced formation of this enzyme(s) was confirmed by the following experiments. *E. coli* was grown overnight on glucose as sole carbon source. The cells were harvested by centrifugation and resuspended in 0.15

M KCl. Aliquots of this suspension were used as inocula for growth media containing either 50 mM glucose or 50 mM acetate as sole carbon source. Growth was followed by measuring the absorbancy at 650 mμ. At different time intervals, samples of the growing cells were removed, disrupted by sonication, and assayed for FDPase activity and protein. It may be seen from Figure 1 that the cells grew very rapidly on glucose and that the specific activity of FDPase remained constant or, in fact, decreased during rapid logarithmic growth. Cells placed in acetate medium, however, grew only after a lag period of more than 4 hours. A rapid rise in the specific activity of FDPase preceded growth. The specific activity increased from 3.2 to 8.0 before any growth was detected and reached a maximum in 0.3 of

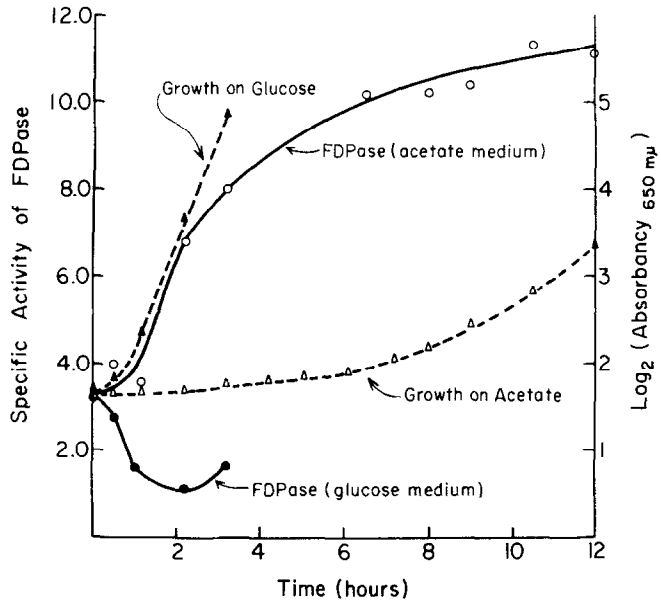

Fig. 1. Effect of carbon source on fructose-1,6-diphosphatase formation and cell growth by glucose-grown E. coli. Cells grown on glucose were incubated at 37° in fresh growth media containing 50 mM acetate or 50 mM glucose as carbon sources. Samples were removed and analyzed for FDPase as described in Table 1. Specific activity of cells in acetate (O—O) and glucose (●—●) media plotted vs. time. Cell density as $\log_2(A_{650\ m\mu})$ of cells in acetate (Δ---Δ) and glucose (▲---▲) media plotted vs. time.

a generation. These results indicate that a preferential synthesis of FDPase occurred during the adaptation of glucose-grown cells to acetate. Formation of the FDPase was probably necessary for growth, since it preceded growth on acetate. In this respect, induction of FDPase is similar to that of isocitratase, one of the key enzymes of the glyoxylate cycle (9).

In a similar experiment, cells grown on acetate as sole carbon source were used as inocula for flasks containing 50 mM acetate, 50 mM glucose, or 50 mM acetate plus 5 mM glucose as carbon sources. Cells placed in the acetate medium continued to form FDPase at a high rate (Fig. 2). Although the cells grew very rapidly on glucose, there was an immediate cessation of new FDPase formation until late in the logarithmic phase, when a small increase occurred. When cells were suspended in a medium contain-

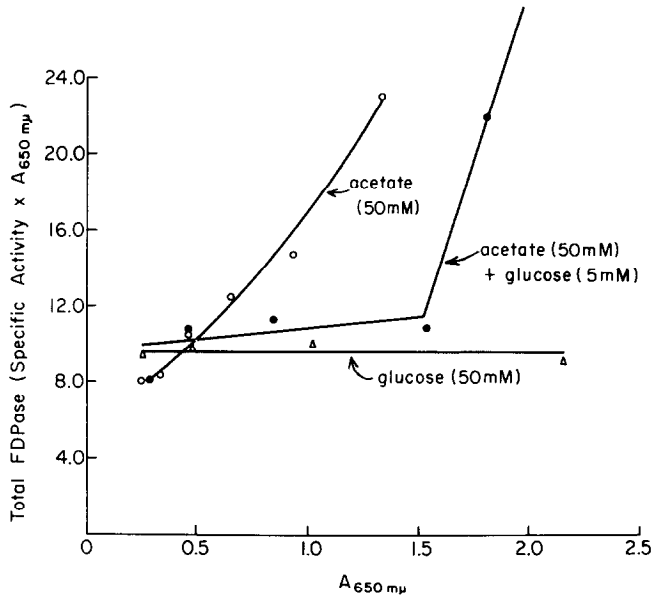

Fig. 2. Effect of carbon source on fructose-1,6-diphosphatase formation by acetate-grown E. coli. Suspensions of acetate-grown cells were incubated in fresh growth media containing 50 mM acetate (○—○), 50 mM acetate plus 5 mM glucose (●—●), or 50 mM glucose (Δ—Δ) as carbon sources. Samples were removed and analyzed for FDPase as described in Table 1. The total enzyme content is expressed as the product of specific activity and cell density.

ing 5 mM glucose and 50 mM acetate, a diauxic growth curve was observed. During the initial growth phase there was a cessation of new formation of FDPase. This was followed by a lag period which preceded a second phase of logarithmic growth and a rapid rise in total FDPase activity. Presumably the formation of new FDPase was repressed by the presence of glucose in the growth medium, as indicated by the diauxic growth curve.

No evidence for the presence of an inhibitor or activator was found upon assaying mixtures of glucose-grown extract and acetate-grown extract in different ratios. There was no detectable enzyme in either glucose or acetate growth medium after removal of cells. The FDPase was completely solubilized by sonication. There were no demonstrable metal or cofactor requirements after either gel filtration through Sephadex G-25 or precipitation with ammonium sulfate. The pH optimum of the acetate-induced FDPase was 5.3, with 53% of maximal activity at pH 7.1 and no activity at pH 9.5.

Studies on the substrate specificity of the acetate-induced enzyme(s) at pH 4.7 showed that FDP and all hexose monophosphates tested were hydrolyzed at about the same rate at a concentration of 10 mM. The rates of hydrolysis of other phosphate compounds relative to FDP were as follows: ribose-5-phosphate, 25%; p-nitrophenyl phosphate, 15%; triose phosphates, 6-phosphogluconate, ATP, and sedoheptulose-7-phosphate, 5% or less. These studies indicate that the induced enzyme(s) could be properly designated as a hexose phosphatase(s). No detectable hydrolysis of sedoheptulose-1,7-diphosphate was observed with extracts from either ribose- or acetate-grown cells. The absence of sedoheptulose-1,7-diphosphatase activity and the presence of high FDPase activity in ribose-grown cells support the obligatory role of FDPase in the conversion of pentose to hexose-6-phosphate in E. coli (10, 11).

At pH 7 in Tris-maleate buffer, the K_m values for the induced phosphatase were 1 x 10^{-3} M with FDP as substrate and 5 x 10^{-3} with glucose-6-phosphate. In order to establish that fructose-6-phosphate was formed from FDP, an acetate-grown extract was incubated at pH 7 with FDP and $NADP^+$ in the presence of excess phosphohexoisomerase and glucose-6-phosphate dehydrogenase. A rapid formation of NADPH occurred, which was dependent upon the addition of FDP, extract , and glucose-6-phosphate dehydrogenase.

The substrate specificity, acid pH optimum, and very low activity at alkaline pH clearly distinguish this enzyme from the induced non-specific alkaline phosphatase observed by Horiuchi (12) and Torriani (13) in extracts of E. coli grown on media containing low levels of phosphate. It is not understood why the activity with hexose monophosphates also increased in the acetate-grown cells. The formation of free sugars may be required for bacterial growth, or, on the other hand, E. coli may produce a phosphatase(s) that is less specific than the enzyme from other sources.

In summary, these results indicate that: high levels of FDPase activity were formed in E. coli only when the growth substrate was a non-hexose; formation of the enzyme(s) preceded growth on acetate; the induced enzyme(s) was most active toward hexose phosphates, had a high affinity for FDP and catalyzed the formation of fructose-6-phosphate from FDP. These findings support the role of the enzyme(s) in glyconeogenesis.

ACKNOWLEDGEMENTS

We wish to thank Drs. S. P. Colowick and O. Touster for their advice and encouragement, and Dr. B. L. Horecker for generous gifts of sedoheptulose-7-phosphate and sedoheptulose-1,7-diphosphate.

This work was supported by Research Grant No. E-3643 from the National Institutes of Health, United States Public Health Service (to Dr. Pogell), and by Research Grant No. G-7590 from the National Science Foundation, U.S.A. (to Dr. Touster).

REFERENCES

1. Krebs, H.A., Bull. Johns Hopkins Hosp., 95, 19 (1954).
2. Pogell, B.M., and McGilvery, R.W., J. Biol. Chem., 208, 149 (1954).
3. Dagley, S., and Dawes, E.A., Biochem. J., 45, 331 (1949).
4. Herbert, D., Microbial Reaction to Environment, Eleventh Symposium of the Society for General Microbiology, Cambridge Univ. Press, Cambridge, England, 1961, p. 391.
5. Kornberg, H.L., and Gotto, A.M., Biochem. J., 78, 69 (1961).
6. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).
7. Gomori, G., J. Lab. Clin. Med., 27, 955 (1941-42).
8. Hofsten, B., Biochim. Biophys. Acta, 48, 171 (1961).
9. Kornberg, H.L., Gotto, A.M., and Lund, P., Nature, 182, 1430 (1958).
10. Gunsalus, I.C., Horecker, B.L., and Wood, W.A., Bact. Rev., 19, 79 (1955).
11. Couri, D., and Racker, E., Arch. Biochem. Biophys., 83, 195 (1959).
12. Horiuchi, T., Horiuchi, S., and Mizuno, D., Nature, 183, 1529 (1959).
13. Torriani, A., Biochim. Biophys. Acta, 38, 460 (1960).