REGULATION OF GLUCONEOGENESIS IN ACETOBACTER XYLINUM BY HEXOSES

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Summary

Cellulose formation in Acetobacter xylinum occurs at a similar rate when either glucose, fructose or succinate are added as the source of carbon. When either glucose or fructose are added in addition to succinate, cellulose formation from succinate is decreased by 80%. A similar inhibition by added hexoses is obtained when the conversion of pyruvate to cellulose is followed. Possible mechanisms correlating these observations with the properties of the enzyme systems involved in the interconversion of the various substrates are discussed.

Gluconeogenesis as expressed by cellulose synthesis during growth of A. xylinum on citrate-cycle intermediates, is subject to control exerted over the synthesis of the pace-maker enzyme pyruvate-phosphate dikinase, which catalyzes the direct phosphorylation of pyruvate to phosphoenolpyruvate (PEP) (1). Utilizable sugars like glucose and fructose repress the synthesis of the dikinase (2). It appeard possible that there exists an additional system for control of gluconeogenesis, beyond the level of enzyme synthesis. Such a control might regulate either directly the activity of pyruvate-phosphate dikinase or function in a more complex manner involving the interaction of various metabolic systems. To examine these possibilities the effect of the exogenous addition of sugars on the fate of the carbon atoms of succinate and pyruvate was investigated in resting cells with an already high pre-existing level of the dikinase. The results obtained indicate clearly that even in the presence of the enzyme an alternative mechanisms exists that regulates gluconeogenesis in this organism.

Materials and Methods. Succinate-grown cells of A. xylinum were grown for 24 hours and harvested as described previously (3).

Incubation procedure. The standard reaction mixture (10 ml) contained 25 -35 mg of cells, dry weight, substrates and 50 mM phosphate buffer (pH 6.0) with oxygen

as the gase phase, in 100 ml stoppered flasks provided with a center-well for CO_2 absorption. 1 ml of 40% KOH was added to to well. The mixtures were shaken at 100 ascillations per min in a water bath at 30^{o}. After 40 min incubation the cells and synthesized cellulose were sedimented by centrifugation and repeatedly washed. Supernatants were combined and kept for analysis. The cellulose initially present was less than 15% of the value found after incubation with the substrates. The endogenous rate of cellulose synthesis was negligible. The rate of cellulose synthesis and CO_2 production with the various substrates tested, was linear for at least 1 hour.

Analytical methods. Cellulose samples were freed from protein, degraded to glucose and determined chemically as previously described (4). ^{14}C-labelled material was counted in a Packard Tri-Carb liquid scintillation spectrometer. Values were corrected for quenching by the use of internal standards. The following compounds were determined enzymatically: acetate with acetokinase (5); acetaldehyde with alcohol dehydrogenase; pyruvate with lactic dehydrogenase; succinate with succinic dehydrogenase (6); fructose with hexokinase, phosphoglucose isomerase, glucose-6 phosphate dehydrogenase system; and glucose with the glucose oxidase-peroxidase system. Gluconate was determined either enzymatically with the gluconokinase, 6-phosphogluconate dehydrogenase system, or colorimetrically (7).

The analytical recoveries of ^{12}C and ^{14}C of added substrates were close to 100% in all the systems tested.

Results. Washed succinate-grown cells of A. xylinum formed cellulose from glucose and fructose at approximately the same rate (Table 1, Exp. 3 and 4). With uniformly ^{14}C-labeled sugars the specific radioactivity of the cellulose carbon was equal to that of the added sugars. The ratio of sugar converted to cellulose to that converted to CO_2 was approximately the same with the two hexoses. With glucose, however, more than 90% of the sugar metabolized was converted to gluconate, whereas with fructose, CO_2 and cellulose were the only detectable products formed.

The rate of cellulose synthesis from succinate was similar to that obtained with the hexoses. With pyruvate as carbon source, considerable cellulose was also obtained, albeit at a rate 1/3 to 1/2 of that obtained with the other substrates (Table 1, Exp. 1 and 2). With uniformly ^{14}C-labeled acids the amounts of substrate carbon incorporated into cellulose accounted for all of the cellulose carbon measured chemically. Cellulose synthesis from pyruvate was accompanied by the

Table 1.

Effect of sugars on gluconeogenesis from pyruvate and succinate

Exp.	Substrates[a]	Cellulose synthesis	Conversion of ^{14}C-labeled substrate to cellulose[b]	$^{14}CO_2$ production (as ^{14}C-labeled substrate)[b]	Utilization of non-sugar substrate[c]	Utilization of sugar substrate[d]
		Micromoles/mg cells/hr				
1.	^{14}C-Pyruvate	0.25	0.54	5.70	10.0(5.1)	-
2.	^{14}C-Succinate	0.67	0.96	3.13	4.2(0)	-
3.	^{14}C-Glucose	0.70	0.68	0.44	-	16.2(15)
4.	^{14}C-Fructose	0.56	0.58	0.30	-	1.0(0)
5.	^{14}C-Pyr + glu	1.31	0.11	13.14	16.0(4.8)	16.7(15)
6.	^{14}C-Glu + pyr	1.33	1.23	0.40		
7.	^{14}C-Pyr + fruc	1.28	0.06	10.50	13.7(5.5)	1.7(0)
8.	^{14}C-Fruc + pyr	1.28	1.22	0.30		
9.	^{14}C-Succ + glu	1.51	0.10	3.40	3.7(0)	17.0(15)
10.	^{14}C-Glu + succ	1.53	1.46	0.50		
11.	^{14}C-Succ + fruc	1.36	0.09	3.60	3.7(0)	1.6(0)
12.	^{14}C-Fru + succ	1.32	1.28	0.30		

(a) Incubated as described in Methods and Materials. Pyruvate and succinate 40 mM; sugars, 30 mM. When stated substrates were uniformly ^{14}C-labeled with a specific activity of 3-5 x 10^3 counts/ min per μmole.
(b) Calculated from total ^{14}C incorporation and the specific activity of labeled substrate.
(c) Values in parentheses refer to C_2 compounds found in the medium (8-10% acetaldehyde with the rest as acetate).
(d) Values in parentheses refer to gluconate found in the medium.

appearance in the medium of considerable amounts of acetate and small amounts of acetaldehyde, which accounted for 50% of the pyruvate utilized. The remaining pyruvate was oxidized to CO_2. With succinate as substrate, however, CO_2 and cellulose were the only products detected and together represented almost 100% of the substrate carbon utilized.

To test the effect of hexoses on gluconeogenesis, cells were incubated with either ^{14}C-labeled pyruvate or succinate and unlabeled glucose or fructose, or with ^{14}C-labeled sugars and unlabeled acids. In all of these experiments (5 - 12, Table 1) the rate of total cellulose synthesis increased to more than twice the rate observed with any single substrate. The cellulose carbon was derived almost exclusively from the sugar component of the mixture. The conversion of the gluconeogenic substrates to cellulose was suppressed by more than 80% in the presence of the sugars. The possibility that this suppression is due to isotopic dilution in a common pool of an intermediate (i. g. hexose phosphate) arising both from the sugars and the gluconeogenic substrates (Cf. 8) appears to be unlikely. Such a dilution would require a ratio between the rates of supply of such an intermediate of far greater disproportion than that indicated by comparing the rate of hexose utilization (Table 1, column VII) relative to the rate of either pyruvate or succinate entry into cellulose (Table 1 columns I and II).

The conversion of sugar carbon to CO_2 was not affected by the presence of succinate or pyruvate, except at lower concentrations of sugars (5 - 10 mM). In the latter situation, addition of pyruvate or succinate resulted in a 50% decrease in the rate of sugar conversion to CO_2, concomitant with a two fold increase in the rate of sugar conversion to cellulose.

The rate of pyruvate utilization increased in the presence of the sugars. The data on the rates of pyruvate consumption, $^{14}CO_2$ production and acetate accumulation indicate that the sugars caused a 1. 5 to 2 fold increase in the rate of pyruvate decarboxylation and a 2 to 3 fold increase in the rate of pyruvate oxidation to CO_2. Succinate oxidation, however was not significantly affected by the sugars.

To examine the possibility that the hexose suppression occured directly on pyruvate-phosphate dikinase, cell-free extracts were analyzed (1, 2) for the effect of hexoses on pyruvate phosphorylation. Glucose and fructose and their phosphate esters did not affect dikinase activity when assayed with purified enzyme preparations. However, the rate of PEP formation from pyruvate in crude extracts was 50 - 60% lower in the presence of 20 mM of the sugars.

Discussion. Six moles of energy-rich phosphate are required to synthesize 1 mole of the hexose-phosphate precursor of cellulose from pyruvate or succinate as compared to 1 mole from glucose or fructose. The high energy costs of gluconeogenesis are met in the cell by the oxidation of the substrates in the citrate cycle (9). Since in A. xylinum pyruvate is an obligatory gluconeogenic intermediate (10), it follows that for each molecule of hexose-phosphate synthesized from succinate there is an intermediate formation of 2 molecules of pyruvate. On this basis the data of Experiment 2 (Table 1) indicate that approximately one of three molecules of pyruvate arising from succinate was converted to cellulose and 2 entered the citrate cycle and were oxidized. (cf. 8, 11). With exogenously provided pyruvate, however, the ratio of substrate oxidized to that converted to cellulose was much higher. This difference between the two substrates as well as the inferiority of pyruvate as a gluconeogenic substrate, could be attributed to the diversion of part of the PEP, arising from pyruvate, from the gluconeogenic pathway to the formation of oxaloacetate mediated by PEP- carboxylase, which in turn is strongly inhibited by succinate (10). The appearance of considerable amounts of acetaldehyde and acetate in the course of pyruvate oxidation but not during succinate oxidation is compatible with the kinetic properties of the pyruvate decarboxylase (12) and the pyruvate-phosphate dikinase systems as will be discussed elsewhere.

The pyruvate phosphorylation system as the initial and rate-limiting step of gluconeogenesis in A. xylinum would make it a potential control point for regulating the gluconeogenic flux in this organism. Of the three enzymes initiating hexose-phosphate formation from the substrates examined here, namely: glucokinase, fructokinase (13) and pyruvate-phosphate dikinase, the latter is most susceptible in inhibition by a fall in the ATP/AMP concentration ratio (1). Since in addition, the sugar kinases are 7-10 times more active in these cells than the dikinase, a reduced rate of pyruvate phosphorylation could be expected to occur in the added presence of the sugars. An additional factor which could affect gluconeogenesis is that the relatively high rate of sugar phosphorylation (which is even enhanced in the presence of pyruvate and succinate, as indicated by the increase in the rate of sugar conversion to cellulose) with the concomitant increase in ADP/ATP concentration ratio would result in an increased activity of pyruvate kinase (14) which in turn would further deprive PEP from the gluconeogenic pathway. The total effect of the sugars on gluconeogenesis could thus be visualized as an indirect end product inhibition of this pathway.

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References

1. Benziman, M. and Palgi, A., J. Bacteriol 104, 211 (1970).
2. Benziman, M. and Eisen, N., J. Biol. Chem. in press.
3. Benziman, M. in Methods in Enzymology (J. M. Lowenstein, ed.) Academic Press, New York, Vol. XIII, p. 129, 1969.
4. Benziman, M. and Burger-Rachamimov, H., J. Bacteriol 84, 625 (1962).
5. Rose, I. A., Grunberg-Manago, M., Korey, S. R. and Ochoa, S., J. Biol. Chem. 211, 737 (1954).
6. Rodgers, K., Biochem. J. 80, 240 (1961).
7. Lien, O. G., Anal. Chem. 31, 1363 (1959).
8. Krebs, H. A., Hems. R., Weidemann, M. J. and Speak, R. N., Biochem. J. 101, 242 (1966).
9. Benziman, M. and Levy, L., Biochem. Biophys. Res. Commun. 24, 214 (1966)
10. Benziman, M., J. Bacteriol 98, 1005 (1969).
11. Exton, J. H. and Park, C. R., J. Biol. Chem. 242, 2622 (1967).
12. Imeral, S. and Benziman, M., Israel J. Chem. 8, 137 (1970).
13. Rivetz, B. and Benziman, M., Israel J. Chem. 8, 148 (1970).
14. Benziman, M., Biochem. J. 112, 631 (1969).