PHOSPHOENOLPYRUVATE CARBOXYKINASE IN GLUCONEOGENESIS AND ITS REPRESSION BY HEXOSES IN YEASTS*

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An ADP-dependent phosphoenolpyruvate carboxykinase (PEPCK) which can form oxaloacetate from phosphoenolpyruvate and CO_2 was described in bakers' yeast by Cannata and Stoppani (1963a, 1963b), who ascribed to it the essential physiological role of oxaloacetate synthesis (see also Cazzulo and Stoppani, 1965). Doubts concerning this interpretation arouse, however, when it was later shown that this microorganism contains also pyruvate carboxylase, which carries out the carboxylation of pyruvate to oxaloacetate in the presence of ATP (Losada et al., 1964; Gailiusis et al., 1964, Cazzulo and Stoppani, 1965). Recent work from our laboratory (Ruíz-Amil et al., 1965) strongly suggested that pyruvate carboxylase is involved in the carboxylation of pyruvate to oxaloacetate whereas PEPCK is responsible for the decarboxylation of oxaloacetate to phosphoenolpyruvate. It was found by growth of two yeast species (Rhodotorula glutinis and Hansenula anomala) on different carbon sources that the level of PEPCK is high when gluconeogenesis is active (cells grown on acetate, pyruvate, malate or aspartate) and low when glycolisis is

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operative (cells grown on glucose). Polakis and Bartley (1965) estimated the activity of PEPCK in another yeast (Saccharomyces cerevisiae) grown on different carbon sources but could not present quantitative results.

This communication presents evidence indicating that PEPCK is involved in gluconeogenesis in yeast and that its formation is repressed by hexoses rather than induced by oxaloacetate precursors.

METHODS - Rhodotorula glutinis (RH-1413) and Hansenula anomala (W-10) were grown aerobically with vigorous shaking at 30° C on the synthetic medium of Olson and Johnson (1949) but with one of the following carbon sources: 0.1 % hexose (glucose, fructose or galactose); 0.2 % aspartate; 0.1 % glucose plus 0.2 % aspartate. When ammonia was excluded from the medium, aspartate was used as the only nitrogen source. During the logarithmic phase, the cells were harvested by slow speed centrifugation and washed with 0.05 M Tris-HCl, pH 7.6. The crude extracts were prepared in the cold by grinding the cells in a mortar with twice their weight of alumina and extracting with three times their weight of the same buffer. After centrifugation for 20 minutes at 20,000 x g, PEPCK was assayed in the supernatant by measuring $^{14}CO_2$ fixation under essentially the conditions described by Cannata and Stoppani (1963a), but in the presence of lactate dehydrogenase and NADH to avoid interference by pyruvate carboxylase (Losada et al., 1964). The reaction mixture was incubated at 30° C for 30 minutes and included in a final volume of 2 ml: cell-free extract containing 0.1 - - 0.8 mg protein, 4 units of glutamate-oxaloacetate transaminase, 4 units of lactate dehydrogenase, and the following in micromoles: citrate-phosphate buffer, pH 5.2, 250; ADP, 10; PEP, 10; $KH^{14}CO_3$, 10 (6 μC); NADH, 1; $MnCl_2$, 20; glutamic acid, 30. $^{14}CO_2$ fixation was determined by measuring the radioactive carbon in stainless steel planchets with a thin window counter of approximately 5 % efficiency. Pyruvate carboxylase and malic enzyme were

estimated as described previously (Ruiz-Amil et al., 1965). Protein was estimated by the methods of Lowry et al. (1951). Enzyme units are expressed as micromoles of CO_2 fixed per minute.

RESULTS - As can be see in Table I, extracts of R. glutinis or H. anomala grown on glucose alone or plus aspartate

TABLE I

LEVELS OF PHOSPHOENOLPYRUVATE CARBOXYKINASE IN YEASTS GROWN WITH AND WITHOUT GLUCOSE

Carbon source	R. glutinis	H. anomala
	milliunits per mg protein	
Glucose	0.2	0.1
	0.3	0.1
Glucose plus aspartate	0.4	0.1
	0.5	0.1
Aspartate	4.2	12.3
	5.0	4.6

showed levels of PEPCK activity 10-100 fold lower than comparable extracts from cells grown on aspartate in the absence of glucose. Since aspartate can enter the cells and serve as the only nitrogen source when glucose is present in the medium (Fig. 1), the low level of PEPCK observed in cells grown on the mixture of glucose and aspartate may be ascribed to the repression of its synthesis by glucose. It thus appears that glucose is the nutritional repressor, rather than aspartate the inducer, of PEPCK.

Cells grown on either fructose or galactose behaved as those grown on glucose with respect to the level of PEPCK, the extracts showing again in these cases low specific activities of the order of 0.1 milliunits per mg of protein.

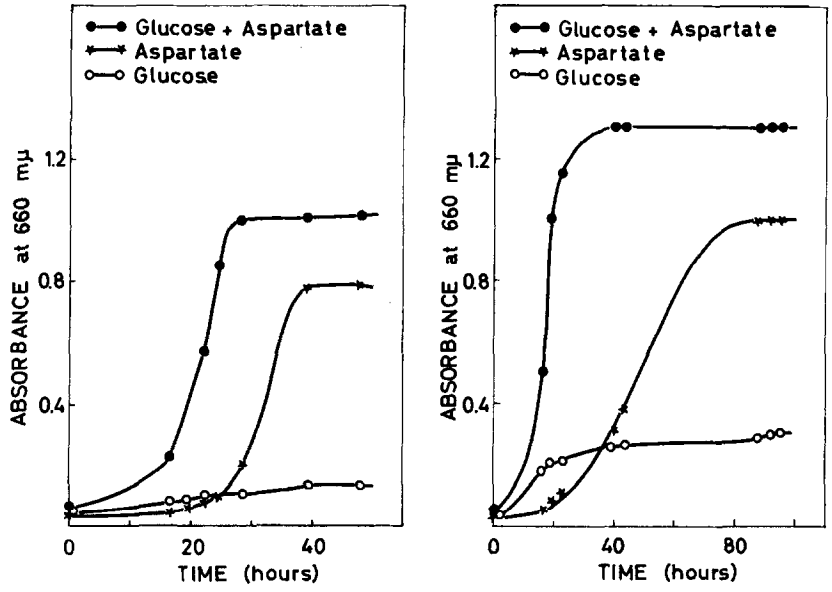

Figure 1. Growth curves of R. glutinis (left) and H. anomala (right) in the absence of ammonia with glucose and (or) aspartate as indicated.

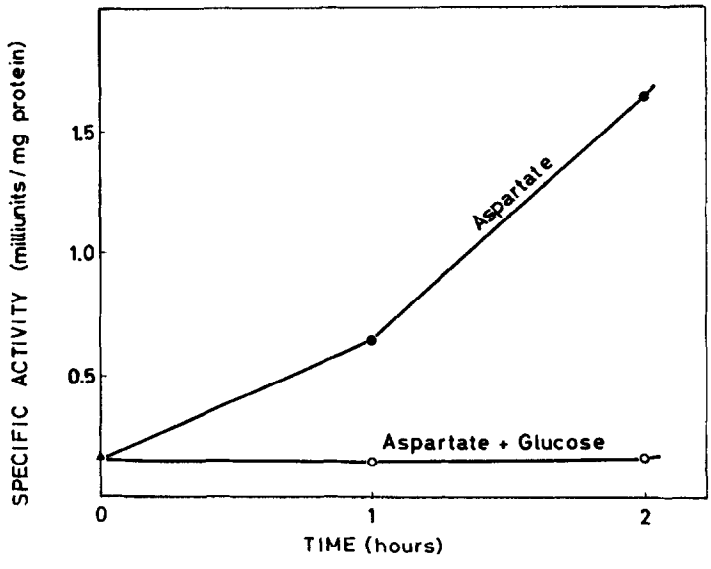

Figure 2. Derepression of PEPCK in R. glutinis. At zero time the yeast cells grown on glucose were transferred into a medium containing aspartate or aspartate plus glucose. The specific activity of PEPCK was then determined at the times indicated as described under Methods.

The initial kinetics of the derepression of PEPCK is shown in Fig. 2. Cells of R. glutinis grown on glucose and harvested during the logarithmic phase were washed and transferred at zero time to a medium containing aspartate or a mixture of aspartate and glucose. It can be seen that the formation of the enzyme is repressed by glucose.

We have found that on changing from a glucose to a pyruvate medium, H. anomala can grow perfectly well without forming detectable amounts of malic enzyme, the level of PEPCK rising markedly (about 10 fold). In these cells grown on pyruvate, the specific activity of pyruvate carboxylase reached an average value of 80 milliunits per mg protein. These results show that, in H. anomala, malic enzyme is neither involved in the carboxylation of pyruvate nor essential for gluconeogenesis from this ketoacid, and complement previous findings from our laboratory (Ruíz-Amil et al., 1965) leading to the conclusion that the function of malic enzyme in yeast is to decarboxylate malate to pyruvate.

Our present evidence indicates that, in yeast, pyruvate carboxylase is indispensable for the carboxylation of pyruvate, and PEPCK for the formation of phosphoenolpyruvate from oxaloacetate.

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