Effect of Glucose on Pyruvate Utilization by *Rhodotorula glutinis*

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Summary. The utilization of glucose and pyruvate by the yeast *Rhodotorula glutinis* in a medium containing both carbon sources has been studied. Glucose is readily consumed whereas the uptake of pyruvate is completely blocked by the presence of the sugar.

The content of pyruvate kinase and phosphoenolpyruvate carboxykinase in *R. glutinis* cells growing on glucose plus pyruvate are drastically affected with time by the disappearance of the sugar from the culture medium. After complete exhaustion of glucose, the level of pyruvate kinase drops sharply down to a minimum whereas that of phosphoenolpyruvate carboxykinase rises abruptly up to a maximum.

Feeding experiments with labelled compounds show that glucose affects the utilization of the amino acids alanine and aspartate, and conversely that the amino acids influence the utilization of the sugar. Glucose breakdown and its incorporation into polysaccharides is controlled by the amino acids and gluconeogenesis from the amino acids is controlled by the sugar.

In previous work from this laboratory (RUIZ-AMIL et al., 1965; TORRONTÉGUI et al., 1966; FERNÁNDEZ et al., 1967) it was shown that glucose was both the nutritional inducer of pyruvate kinase and the repressor of phosphoenolpyruvate carboxykinase in the yeast *Rhodotorula glutinis*. As in other organisms, the function of pyruvate kinase was to form pyruvate from phosphoenolpyruvate during glycolysis and that of phosphoenolpyruvate carboxykinase was to form phosphoenolpyruvate from oxaloacetate during gluconeogenesis; the reverse reactions do not play probably any physiological role. Pyruvate was converted into phosphoenolpyruvate via oxaloacetate by the combined action of pyruvate carboxylase and phosphoenolpyruvate carboxykinase. The physiological function of the yeast malate enzyme, which was repressed not only by glucose but also by acetate, was to catalyze the TPN dependent reductive decarboxylation of malate to pyruvate but not to provide malate from pyruvate.

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We found also (FERNÁNDEZ et al., 1967) that, under conditions in which R. glutinis cells were growing in a medium containing glucose and aspartate, the amino acid could be simultaneously utilized with the sugar even if its gluconeogenic role was hindered as a consequence of the repression of phosphoenolpyruvate carboxykinase by the hexose. Once glucose was exhausted from the medium, the cells continued growing solely on the remaining aspartate and an alteration in the levels of the enzymes discussed above occurred, leading to new metabolic sequences.

Following this approach of using growing yeast cells as a simple and easily modifiable cellular system ready for active glycolysis and gluconeogenesis, we have now carried out experiments with labelled compounds in order to try to elucidate the dubious but fundamental question as to whether the cell economy can support the simultaneous function of these two opposite processes. In particular we have centered our attention on the utilization of glucose and pyruvate, in order to determine firstly whether pyruvate could be concomitantly used with glucose by the yeast cells, and secondly how pyruvate or some of its immediate precursors would influence glycolysis from glucose and, reciprocally, how glucose would influence gluconeogenesis from pyruvate.

The results to be reported in this paper indicate a control by glucose of the entry of pyruvate into R. glutinis cells and confirm previous findings of the induction of pyruvate kinase and the repression of phosphoenolpyruvate carboxykinase by the sugar. Moreover, they present evidence of the existence of control mechanisms for both glycolysis and gluconeogenesis in this yeast.

Material and Methods

Culture of the Yeast

*Rhodotorula glutinis* (strain 1413), kindly supplied by the Departamento de Fermentaciones Industriales, C.S.I.C., Madrid, was grown aerobically with vigorous shaking at 26 °C using the synthetic growth medium of OLSON and JOHNSON (1949), but with one of the following carbon sources: glucose, pyruvate, alanine, aspartate, glucose plus pyruvate, glucose plus alanine or glucose plus aspartate, at the concentration described in each case. The growth of the yeast was determined by measuring the turbidity in a Klett-Summerson colorimeter using filter 66.

Preparation of the Cell-Free Extracts

The yeast cells were harvested at the logarithmic phase by slow-speed centrifugation and washed with 0.9% (w/v) KCl. The crude extracts were prepared in the cold by grinding the fresh cells in a mortar with twice their weight of alumina and by extracting afterwards with twice their weight of 0.05 M Tris-HCl (pH 7.6). After centrifugation for 20 minutes at 20,000 × g the supernatant layer was used as such for determining the activity of the enzymes.