PULSATING GLUCOSE FLUX IN YEAST

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There have been a few recent reports on the periodic fluctuation of glycolytic velocity in yeast. It has been observed that under certain conditions of aerobic-anaerobic transition most glycolytic intermediates beginning from G6P start oscillating sinusoidally around a mean value (Hommes, 1964; Ghosh and Chance, 1964; Betz and Chance, 1965). These studies have demonstrated the operation of concerted feedback regulation at multiple loci of a metabolic sequence and their mutual phase relationship. In view of the lack of a suitable explanation for the control of glucose utilization in yeast, and the expected metabolic coupling between glucose and G6P, we have looked into the short-term kinetics of glucose utilization by yeast cells under aerobic conditions. This communication is a brief report of the oscillatory kinetics of glucose uptake and utilization by intact yeast cells observed in these experiments.

METHODS

A hybrid yeast Saccharomyces fragilis x Saccharomyces dobzhanskii, obtained through the kind courtesy of Prof. H. O. Halvorson, was used in most of these studies. It was maintained on agar slopes containing glucose (2%), peptone (1%) and yeast extract (0.3%). Cells were harvested after 18 hour growth on the above liquid medium at 30 C on a rotary shaker and washed with 150 mM KCl. In order to avoid variation in the degree of aerobiosis of the cells, the washed cells were routinely aerated for an hour

in buffered KCl medium (50 mM triethanolamine, pH 7.2 in 150 mM KCl) before use. For aerobic incubations a stream of gas (95% O_2 - 5% CO_2) was bubbled through the cell suspension. Samples for analysis were withdrawn at intervals and added to a final concentration of 0.5 N perchloric acid. In experiments where sampling intervals were shorter than 10 seconds, the time of sampling was marked with a micro-switch coupled to an event-marker pen on a Bausch and Lomb VOM-5 recorder. Glucose was determined with the glucose oxidase-peroxidase method, inorganic orthophosphate by the method of Fiske and Subbarow and other metabolites by fluorometry (Maitra and Estabrook, 1964). Entry of glucose into the cell is indicated here as "uptake" and the intracellular consumption as "utilization".

RESULTS

Fig. 1 describes an experiment showing the pulsed utilization of glucose from the medium and the accompanying profile of some metabolites linked to early stages of glucose breakdown. After an initial lag the utilization of glucose starts suddenly and then falls off all by itself, after which the process starts again with another spurt of glucose metabolism. The duration of the active and quiescent periods of glucose utilization is 75 seconds through all the four cycles of observation. The rates of glucose disappearance during the active period of the cycles are, respectively, 19.4, 17.2, 16.2 and 16.2 μmole glucose per min per g wet yeast, while the inhibited rates are less than 10% of these values. The reality of the oscillatory kinetics is subscribed by the observation that a plot of the extinction of the acid extracts at 260 mμ against time is linear and the scatter of the data bears no correlation whatsoever with the glucose versus time plot.

Analyses of the G6P and aldolase metabolites show that while there is no clear-cut relationship between their levels and the rates of glucose utilization, the inverse relationship between FDP and the triose phosphate on one hand, and G6P on the other, is predominant. It is apparent that phosphofructokinase is controlling the utilization of G6P. What is curious

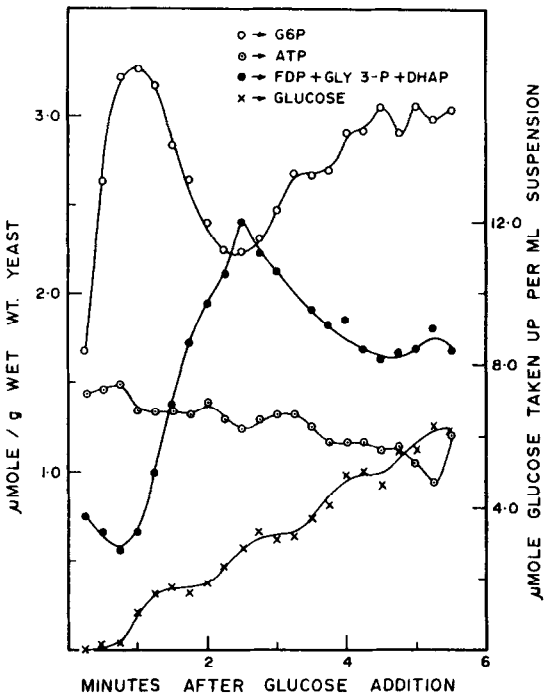

Figure 1. Kinetics of changes of glycolytic metabolites during aerobic glucose utilization by Saccharomyces fragilis x Saccharomyces dobzhanskii. The initial glucose concentration was 13 mM and the cell concentration 117 mg wet weight per ml reaction mixture. The values of glucose refer to the sum of extracellular and intracellular glucose expressed as an average of the entire suspension, and the phosphorylated metabolites as per unit weight of the yeast. G6P refers to glucose 6-P, ATP to adenosine triphosphate, FDP to fructose 1, 6-P, GLY 3-P to glyceraldehyde 3-P and DHAP to dihydroxyacetone P. The values of glucose have been plotted after subtracting the observed from the initial levels and the summation of FDP, GLY 3-P and DHAP has been expressed as C_3 units.

here is that in spite of wide variations in the rate of glucose utilization, the level of G6P affords no indication of the flux through hexokinase. In fact, of all the metabolites measured, only the adenine nucleotides bear certain temporal correlation with the glucose utilization rate. As shown here, every burst of glucose utilization coincides with a simultaneous fall in the ATP level. When the utilization rate slows down, the level of ATP either tends to increase or is prevented from decreasing. Separate experiments

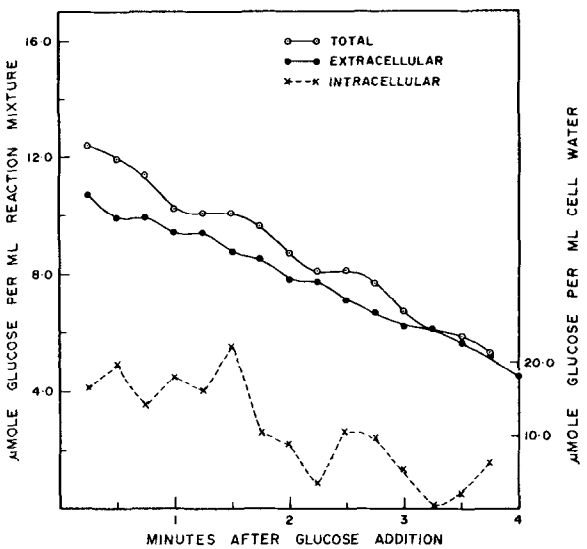

Figure 2. Glucose uptake and utilization by Saccharomyces fragilis x Saccharomyces dobzhanskii. The extracellular glucose was determined by filtration of 0.5 ml aliquots through membrane filters (0.45 μ; Millipore) placed over a series of filtration tubes with perspex funnels and connected to a fast vacuum pump. The filtration time was 2 to 3 seconds for the entire 0.5 ml to come through, about three-fourths of the filtrate being collected in the first second. At the same instant two samples were withdrawn from the incubation mixture (138 mg wet yeast per ml), one put on the filter and the other into acid for determining the extracellular and the total glucose, respectively. The sampling intervals were 15 sec. The extracellular glucose was corrected for the volume of yeast cells in the suspension and the intracellular glucose, shown on the right-hand ordinate, was computed from the total and the extracellular glucose after accounting for 75% as the cell water.

confirm this and reveal an inverse relationship between the rate of glucose utilization and the level of ADP and AMP.

The observed oscillation in the over-all disappearance of glucose from the medium could be due either to a fluctuating hexokinase activity or a varying intracellular pool of glucose brought about by some oscillatory element in the transport process itself. An attempt was made to derermine both the intracellular and the extracellular glucose during such an experiment, so that any such alternation in the rate of entry would be revealed in the analyses of extracellular glucose. These results shown in Fig. 2, indicate

that not only is the rate of glucose disappearance changing periodically, but the rate of entry of glucose into the cells is oscillatory, increasing and decreasing with time. The intracellular concentration of glucose, found by difference, is not constant, but vary rather erratically instead. The point of interest is that during the first cycle of glucose utilization the intracellular concentration of glucose is not below 10 mM (μmole per ml cell water), which should be high enough to saturate the hexokinase (Km for glucose is 0.2 mM at 1.0 mM ATP). It should be pointed out, however, that at no time was it possible to detect the presence of free glucose in the cell whenever the filtered cell pellet was examined directly.

Such oscillation in the rate of glucose utilization has been observed also in Saccharomyces cerevisiae and Escherichia coli strain B and appears to be of a wide dispersion.

DISCUSSION

The results reported here demonstrate that the rates of uptake and utilization of glucose into the yeast cell can depart appreciably from linearity. An explanation of such rhythmic flows of metabolism, consistent with established concepts of control, is negative feedback. It can be visualized that some product(s) of glucose metabolism acts reversibly on either the transport or the utilization process or both and thus controls the rate of flow through the succeeding metabolic steps. It has been found, however, that there is no constant time relationship between the entry and the utilization processes as far as the time periods of each cycle are concerned. It is likely, in view of this variable phase difference, that the proposed feedback metabolite(s) acts separately on the transport and the utilization processes.

In the search for the modulator chemical controlling glucose utilization, the following metabolites have been assayed: all the glycolytic intermediates, isocitrate, α-ketoglutarate, malate, oxaloacetate, glutamate and aspartate. No correlation has been found between the rate of glucose

utilization and the instantaneous level of the above metabolites. In view of the data in Fig. 2, it is unlikely that the intracellular level of glucose is a significant determinant. As indicated earlier, the only metabolites found to change in unison with the glucose utilization rate are the adenine nucleotides. Quantitatively however, this does not appear important if cooperative effects are not invoked; thus it has been observed in other experiments (not shown here) that when glucose utilization rate increases by a factor of 10, the level of ADP and AMP decreases by only 20%. Hommes (1965) had observed that glucose is utilized in an oscillatory manner in phase with the reduction of NAD following functional anaerobiosis in Saccharomyces carlsbergensis. In this case, however, the pulsation in glucose utilization arises independently of the redox state of NAD as observed by fluorometry with the intact cells.

SUMMARY

The entry and the utilization of glucose by washed cell suspension of Saccharomyces fragilis x Saccharomyces dobzhanskii are oscillatory. The duration of the stimulated and inhibited periods is variable and ranges from 10 to 45 seconds at 25 C. No obvious phase relationship between the rates of uptake and utilization of glucose has been observed. It is suggested that the entry into and utilization of glucose by the yeast cell is controlled by feedback through some product(s) of glucose metabolism.

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