A STUDY OF THE FACTORS CAUSING A DECREASE IN THE RATE OF PHOSPHATE UPTAKE BY YEAST DURING PHOSPHATE ACCUMULATION

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SUMMARY

The decrease in the rate of P-absorption developing during P-accumulation by yeast is accompanied by a parallel decrease in glycolysis. Addition of magnesium ions diminished these effects partly suggesting that one of the causes of the decrease in the rate of phosphate absorption is an increasing lack of free magnesium ions in the cell. The possibility that the cellular orthophosphate concentration affected also the rate of P-entry was considered, too.

1. INTRODUCTION

The rate of phosphate absorption by yeast decreases during the accumulation process (BORST PAUWELS 1967). This decrease is considerably more rapid after long preincubation of the yeast in the presence of glucose than after short preincubation periods. Only a small portion of the decrease observed could be ascribed to release of phosphate from the cell. Thus, the rate of entry itself appeared to be affected. The causes of this decrease in the rate of phosphate absorption are investigated. Parallel determinations were made of P-uptake, glycolysis, respiration, ATP-concentration and orthophosphate content of the cells during accumulation of phosphate. Glycolysis is according to GOODMAN & ROTHSTEIN (1957) the main source of energy for phosphate uptake, while ATP is the most important donor of chemical energy in the cell. Orthophosphate might have some regulating function in phosphate uptake. HOLZER (1953) found that the level of orthophosphate in the cell decreased initially to a certain level before the rate of absorption reached a maximum value.

We have also investigated what happens when Mg^{++} ions are added to the yeast together with phosphate. Mg^{++} lessens the decrease in the rate of absorption. The total extent of uptake is much greater in the presence of this ion than in its absence (BORST PAUWELS 1962).

2. EXPERIMENTAL

Saccharomyces cerevisiae, Delft 2, deficient in phosphate, was preaerated without substrate during several days at 25° in a 0.1 M succinate buffer, pH 4.5, containing 9.4 mM KCl and 39 mM $(NH_4)_2SO_4$ in order to decrease the concentration of internal substrate.

Uptake of phosphate was determined by adding radioactive phosphate (^{32}P) to the yeast suspension. For concentrations and conditions, see under results. The uptake of phosphate was stopped by water suction filtration of the radioactive suspension through a Hirsch funnel provided with a filter paper of 2.7 cm

diameter of Schleicher and Schüll No. 602 H. The filter papers were washed twice with 4 ml buffer at 0°C containing 3 % glucose and once with 4 ml of water-free acetone. The radioactivity on the filter papers was determined with a Philips scintillation counter containing a ß crystal of 4 cm diameter.

Analyses of the orthophosphate content of the cells were made by a modification of the method of WAHLER & WOLLENBERGER (1957) after washing the filter papers with the succinate buffer. This modification will be published elsewhere.

Determinations of the ATP concentration were made after extraction of the cells with 5 % $HClO_4$ at 40° during 15 minutes (ESTABROOK & MAITRA 1962) by a fluorimetric method based upon the reduction of NADP to $NADPH_2$ in the presence of hexokinase, glucose-6-phosphate dehydrogenase, glucose and Mg^{++} at pH 7.5 (GREENGARD 1962). Appropriate corrections for glucose-6-phosphate initially present were made.

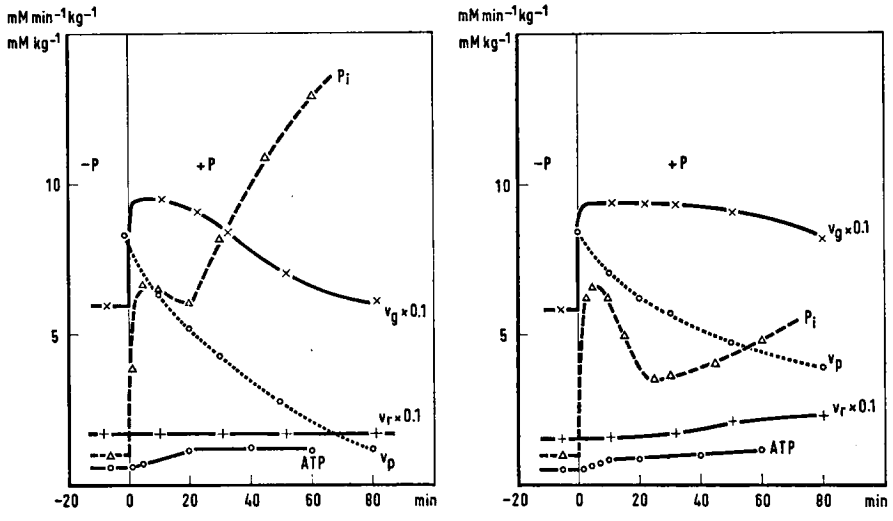

Fig. 1. Rates of glycolysis, respiration and phosphate entry and concentrations of cellular orthophosphate and ATP during accumulation of phosphate in yeast

v_P represents the rate of radioactive phosphate entry expressed in mM min^{-1} per kg fresh weight of yeast. v_G and v_R are the rates of glycolysis and respiration respectively expressed in mM $min^{-1}kg^{-1}$. P_i and ATP are concentrations of orthophosphate and ATP respectively. Both concentrations are expressed in mM kg^{-1}.

The yeast (1 % w/v) was preaerated for 2 hours in the presence of 3 % glucose. Inactive phosphate was then added to a concentration of 3 mM. The uptake of phosphate was measured by adding carrier free radioactive phosphate at appropriate times after addition of the inactive phosphate and taking yeast samples at time intervals of 1–3 minutes. The rates of entry were calculated from the initial slopes of the uptake curves. The yeast concentration was 0.1 % during the determinations of the rates of glycolysis, respiration and P-uptake and 0.5 % during the determinations of orthophosphate and ATP contents.

Fig. 2. Rates of glycolysis, respiration and phosphate entry and concentrations of cellular orthophosphate and ATP during accumulation of phosphate in yeast in the presence of Mg^{++}.

The magnesium was added as 1 mM $MgSO_4$ together with the inactive phosphate. For further details, see legend to *fig. 1.*

The rates of glycolysis and respiration were determined by common manometric methods. Glycolysis is taken to be the production of CO_2 minus 2/3 of the O_2 consumption.

3. RESULTS

The courses of glycolysis, respiration, rate of entry of radioactive phosphate, orthophosphate content of the cell and ATP concentration during accumulation of inactive phosphate are shown in *fig. 1*. The conditions of the experiments are described in the legend of the figure. The rate of entry can be appreciably higher than the rate of net uptake, because of the occurrence of P-release (BORST PAUWELS 1967). It is seen that this rate decreased also with time. Glycolysis increased initially after addition of inactive phosphate to the yeast sus-

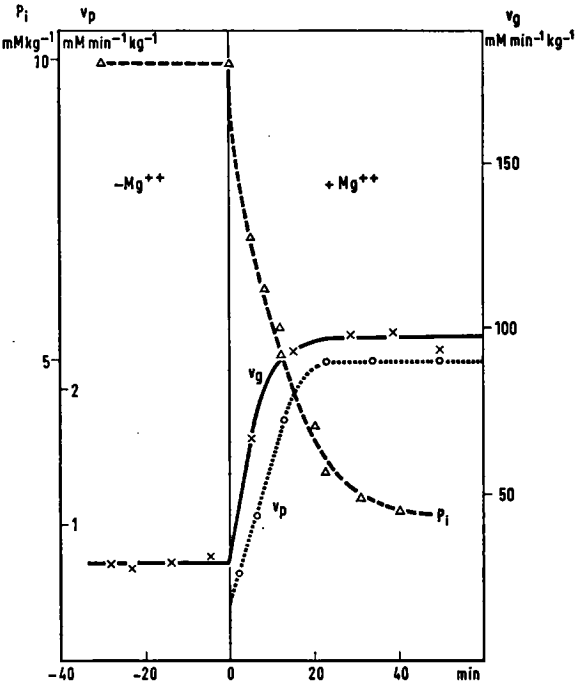

Fig. 3. Reversal by addition of magnesium ions of the inhibition of phosphate uptake and glycolysis and the decrease in the level of cellular orthophosphate developed during phosphate accumulation.

1 % w/v yeast was preaerated for 3 hours in the presence of 3 % glucose followed by the addition of phosphate to the yeast suspension to a concentration of 10 mM. This suspension was centrifuged down after 50 minutes and reincubated in pH 4.5 buffer containing 1 mM phosphate and 3 % glucose. This yeast had a low rate of both glycolysis and phosphate entry due to the accumulation of phosphate. $MgSO_4$ was added 40 minutes later (zero time in the figure) to a concentration of 1 mM. The rate of P-entry was determined by adding radioactive carrier free phosphate together with Mg^{++} and determining the slope of the uptake curve at appropriate times. Thus the radioactive phosphate was added only once in this experiment. Glycolysis was determined after diluting the yeast to 0.2 % w/v.

pension and decreased thereafter. Respiration however did not change much on addition of phosphate. The concentration of cellular orthophosphate increased very rapidly to a peak and decreased for a short time after which it rose again. The ATP concentration increased appreciably after addition of phosphate.

The effect of adding 1 mM Mg^{++} together with the phosphate is shown in *fig. 2.* The decreases in both the rate of P-entry and the rate of glycolysis were much smaller now. The orthophosphate concentration again showed a large initial increase, but it was followed by a much larger decrease than found in the absence of Mg^{++}. The subsequent increase was smaller, too. The concentration of ATP increased more gradually when phosphate and magnesium were added together, than when Mg^{++} was omitted.

The effect of Mg^{++} ions upon glycolysis, P-entry and P_i-level appeared to be reversible as shown in *fig. 3.* Yeast was used which had accumulated phosphate for 90 minutes after a preincubation period of 3 hours in the presence of 3% glucose. As a consequence, the rates of P-entry and glycolysis were low and the cellular orthophosphate concentration was high. Addition of 1 mM Mg^{++} gave rise to a decrease in the orthophosphate level and to an increase in both the rates of P-entry and glycolysis.

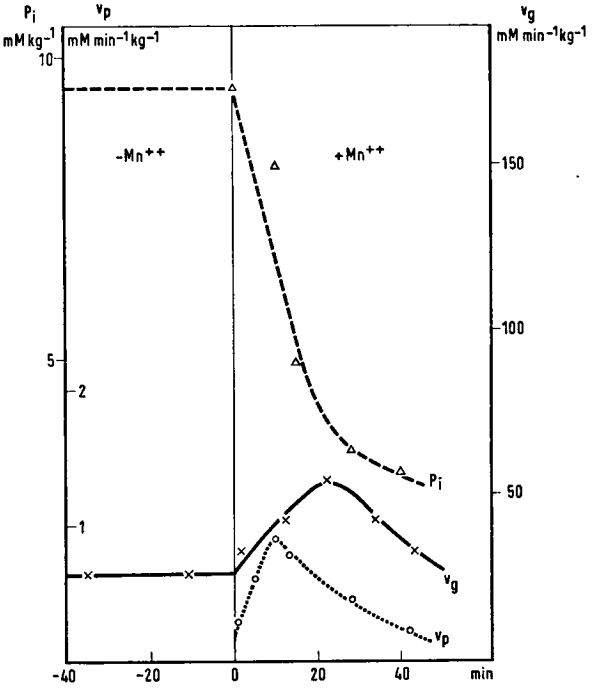

Fig. 4. Effect of Mn^{++} upon the rates of P-entry and glycolysis and upon the level of cellular orthophosphate in yeast which had previously accumulated phosphate.

The yeast was pretreated as described under *fig. 3.* $MnSO_4$ instead of $MgSO_4$ was added to a concentration of 1 mM together with the radioactive phosphate.

We found earlier that Mn^{++} ions did not cause an effect similar to Mg^{++} on phosphate uptake (BORST PAUWELS 1962). Therefore, we have investigated whether 1 mM Mn^{++} does not affect glycolysis either. It appeared that both P-entry and glycolysis were stimulated only for a short time as seen in *fig. 4*. The small initial stimulation of phosphate absorption was apparently overlooked in our previous study. The decrease in the orthophosphate content of the cell after the addition of Mn^{++} was not reversed, however, when the rate of P-entry decreased again.

Finally we carried out an experiment in which the time of preincubation was changed from 50 minutes to 240 minutes. It was found that the decrease in the rate of P-entry depends largely upon the time of preaeration in the presence of 3% glucose (BORST PAUWELS 1967). It is seen from the data in *table 1* that the rate of glycolysis also decreased much more rapidly when phosphate was added after 240 minutes preincubation than after 50 minutes.

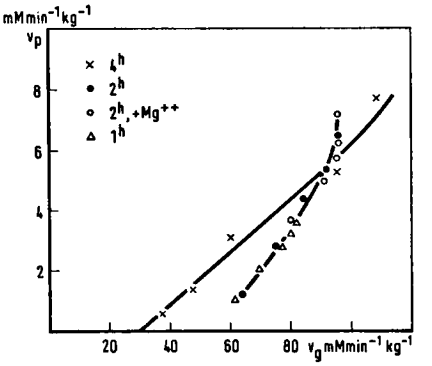

Fig. 5. A plot of the rate of P-entry versus the rate of glycolysis found during accumulation of phosphate by the yeast following different preincubation times. Data from *fig. 1*, *fig. 2* and *table 1*.

Table 1. The rate of entry of radioactive phosphate and the rates of glycolysis and respiration during phosphate accumulation observed after different times of preincubation of the yeast in the presence of 3% glucose

Preincubation time, minutes	50			240		
Quantities involved	v_p	v_g	v_r	v_p	v_g	v_r
Value before P-addition	–	60	14	–	60	14
Value at zero time	4.4	–	–	9.9	–	–
Value 10′ after P-addition	3.7	80	18	7.9	111	18
Value 50′ after P-addition	2.0	68	20	1.4	49	20
Value 80′ after P-addition	1.0	61	26	0.5	38	18

Yeast (1% w/v) was preincubated for 50 or 240 minutes in the presence of 3% glucose. Inactive phosphate was added to a concentration of 3mM (zero time) and the yeast was diluted ten times. Carrier free radioactive phosphate was added at appropriate times after the addition of inactive phosphate in order to determine the rate of entry of radioactive phosphate (v_p) at different stages of phosphate accumulation. v_g is the rate of glycolysis and v_r is the rate of respiration. v_p, v_g and v_r are expressed in mM min^{-1} kg^{-1}.

A plot of the rate of P-entry versus the rate of glycolysis is made in *fig. 5*. A linear relationship exists between both processes except at the higher rates of glycolysis. The slope of the line found after 240 minutes deviates from the slope of the straight line found after the shorter preincubation periods.

4. DISCUSSION

A parallel relation appeared between the rate of P-entry and rate of glycolysis in all cases investigated. A decrease in glycolysis was accompanied by a decrease in the rate of P-absorption and vice versa. No such relation was found with the ATP concentration in the cell. This suggests that it was not a lack of energy which slowed the rates of phosphate entry or glycolysis during P-uptake. The effect of Mg^{++} indicates that a shortage of free Mg^{++} may develop during P-uptake. This might be due to binding of this ion to polyphosphates formed (LISS & LANGEN 1962) or to nucleic acids. A lack of intracellular free Mg^{++} will lead to an inhibition of all the enzymes which require this ion as an activator e.g. the kinases. It is clear that this would result in a decrease in glycolysis. The absence of a decrease in the rate of respiration is not unexpected. The K_m for the enzymatic conversion of pyruvic acid to acetylcoenzyme A is about ten times lower than the K_m for the formation of acetaldehyde, see HOLZER & GOEDDE (1957). Therefore, respiration will be much less affected by a decrease in the rate of glycolysis than the formation of alcohol.

Possibly the partial inhibition of glycolysis is the direct cause of the decrease in the rate of P-entry. This would mean that phosphate absorption is closely linked to glycolysis. Another possibility, however, is that the lack of Mg^{++} ions affects P-transport directly. This view is not in contradiction to our previous observation that Mg^{++} does not have an effect upon the initial rate of P-uptake (BORST PAUWELS 1962). This was concerned only with the action of Mg^{++} at the exterior of the cell membrane. The initial cellular Mg^{++} concentration, however, was not changed in those experiments.

Possibly the level of cellular orthophosphate regulates also the rate of phosphate entry as already suggested by HOLZER (1953). Roughly, the P_i-level in the cell increased when the rate of P-entry decreased as seen in *fig. 1* and *fig. 3*. There are, however, large deviations from this rule. The concentration of orthophosphate in the cell increased initially when both phosphate and magnesium were added to the medium as seen in *fig. 2*. A decrease in the P_i-level followed which was not accompanied by an increase in the rate of P-entry, but rather by a decrease in this rate. The difference in the actions of Mg^{++} and Mn^{++} can also not be due to a regulation of P-uptake by the cellular orthophosphate level, see *fig. 4*. In both cases a decrease in the orthophosphate concentration occurred. The stimulating effect of Mn^{++} on P-uptake, however, was only transient, whereas it was permanent for at least 60 min. in the case of Mg^{++} addition. However, again a striking parallel between glycolysis and rate of P-entry was observed. Possibly the action of Mn^{++} was due initially to a liberation of bound Mg^{++} in the cell and later to a competitive inhibition of the stimulation of Mg^{++},

when the Mn^{++} concentration in the cell had considerably increased.

The decrease in the rate of P_i-entry observed in the presence of Mg^{++} during the first 30 minutes after the addition of phosphate (*fig. 2.*) cannot be due to either an increase in P_i content nor to a decrease in glycolysis. Therefore, some additional factors play a role in the regulation of phosphate absorption, besides those already discussed above.

The final remarks concern the observed increase in glycolysis after the addition of phosphate to the medium. The cells used in our experiments had a very low content of orthophosphate, namely, about 1 mM kg^{-1} fresh weight. This level increased appreciably after the addition of orthophosphate as seen in *fig. 1* and *2*. Probably the low cellular orthophosphate concentration was a limiting factor in glycolysis. A dependence of glycolysis upon the orthophosphate concentration in the cell has been shown by WINDISCH, NORDHEIM & NEUMANN (1960) in yeast and by RACKER & Wu (1959) in ascites tumor cells.

Our study emphasizes once again the importance of the metabolic conditions of the yeast in studies of P-uptake, see also BORST PAUWELS (1967). Large differences in results can be found when the yeast is either preincubated for short or for long periods in the presence of substrate. In addition, the importance of adding magnesium in long term experiments with phosphate has been shown.

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