# GLUCOSE-6-PHOSPHATE AS REGULATOR OF MONOSACCHARIDE TRANSPORT IN BAKER'S YEAST

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Received 4 February 1969

Glucose-6-phosphate appears to be bound ($K_{diss}$ = $1.2 \times 10^{-3}$ M) to a part of the monosaccharide transport system in baker's yeast that is not accessible from the outside and, by this binding, impairs both the capacity of the transport system (possibly by reducing the mobility of the carrier) as well as its affinity. The maximum binding capacity for glucose-6-phosphate seems to be about twice that for glucose but there is mutual interference between glucose and glucose-6-phosphate for their (allotopic?) binding sites.

It was suggested by Sols [1] and by Kotyk and Kleinzeller [2] that the regulation of sugar metabolism in baker's yeast (finding its expression in the Pasteur effect) is not a simple inhibition of a metabolic enzyme (like phosphofructokinase in the presence of ATP and higher levels of citrate, aerobically) but is likely to involve also the transport step across the cell membrane which may be viewed as the first interaction of the monosaccharide with the cell.

As reported earlier [3] growth of baker's yeast on different mixtures of glucose and lactate produced cells in different metabolic states, different also in their sugar transport activity. Scanning the differences in the levels of metabolic intermediates and enzyme activities, glucose-6-phosphate and citrate were selected as likely candidates for the role of a modifier in the transport step. Taking the rate of efflux of D-xylose under conditions near saturation as a measure of the capacity of the monosaccharide transport system [4] it was attempted to relate it to the observed intracellular levels of glucose-6-phosphate or citrate in these cultures. The results shown in fig. 1 indicate that the transport capacity varies inversely with the intracellular glucose-6-phosphate level whereas no simple correlation exists between the citrate level and the sugar transport capacity (fig. 2).

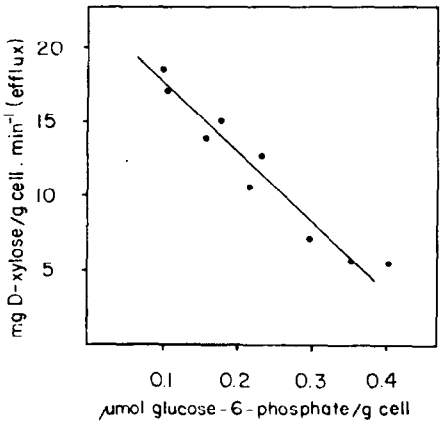


Fig. 1. Dependence of the maximum rate of efflux of D-xylose on the intracellular level of glucose-6-phosphate. D-xylose was estimated with orcinol in samples of cell suspension after preincubation with 10% D-xylose and resuspension in distilled water. Glucose-6-phosphate was estimated with glucose-6-phosphate dehydrogenase and NADP from the increase of absorbance at 340 nm. The regression line of $y$ on $x$ is plotted.

This effect of glucose-6-phosphate cannot be imitated by incubating yeast cells even with high levels of this intermediate (up to 30 mM) and in the presence of fluoride to block phosphatase activity, as

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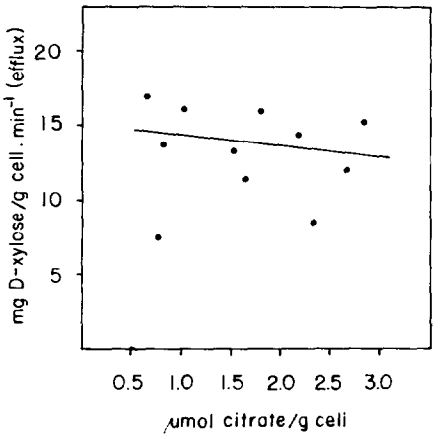


Fig. 2. Relationship between the maximum rate of efflux of D-xylose and the intracellular level of citrate. D-xylose was estimated as in fig. 1, citrate was determined according to ref. [8].

it apparently cannot enter the yeast cell and, for some reason, cannot approach the glucose-6-phosphate sensitive site from the outside.

To answer the possible objection that the glucose-6-phosphate level affects primarily the activity of hexokinase [5] homogenates of baker's yeast grown at different substrate concentrations [3] were prepared with Ballotini glass beads no. 12 and hexokinase activity was assayed both from the disappearance of free glucose from the mixture and from the decrease of high-energy phosphate level. It was found that the activity of hexokinase was nearly the same in

Table 1
Effect of glucose-6-phosphate on hexokinase activity in a baker's yeast homogenate

| Concentration of glucose-6-phosphate (mM) | Hexokinase activity (% of control ± S.E.) |
|---|---|
| 0.01 | 100 |
| 0.4 | 90 ± 7 |
| 2.0 | 67 ± 8 |

The activity was determined from the rate of disappearance of free glucose from the mixture which contained initially 0.2 ml cell-free supernatant (0.08 hexokinase unit), 30 μmol NaF, 3.7 μmol ATP, 20 μmol $MgCl_2$, 80 μmol $NaHCO_3$ and 5 μmol glucose in a volume of 2.5 ml.

the various cultures while the level of glucose-6-phosphate varied considerably. To make sure that the concentration of glucose-6-phosphate in the test medium is not unduly lowered as compared with conditions *in vivo* different concentrations were added to the homogenate to resemble the intracellular concentrations and here again (table 1) no effect on hexokinase activity was found. It was then concluded that glucose-6-phosphate is probably an inhibitor of the transport step itself.

To establish whether glucose-6-phosphate is actually bound to a membrane component, labelled glucose-6-phosphate was prepared by a technique based on that of Betto and Longinotti [6] but using a simplified procedure for the recovery of the product. A re-

Table 2
Equilibrium dialysis of the yeast membrane fraction against labelled glucose and glucose-6-phosphate. The values represent averages of at least three measurements, with 11 mg membrane dry weight.

| Ligand | Concentration (M) | Inhibitor | Ratio bound/free | Apparent $K_{diss}$ ($K_i$) |
|---|---|---|---|---|
| D-glucose-$^{14}$C | $10^{-5}$ | – | 0.31 | |
| | $10^{-4}$ | – | 0.28 | $1.1 \times 10^{-3}$ M |
| | $10^{-3}$ | – | 0.12 | |
| | $10^{-2}$ | – | 0.05 | |
| | $10^{-5}$ | $10^{-2}$ M glucose-6-phosphate | 0.13 | ($7.0 \times 10^{-3}$ M) |
| D-glucose-6-phosphate-$^{14}$C | $5 \times 10^{-5}$ | – | 1.22 | |
| | $10^{-4}$ | – | 1.14 | $1.2 \times 10^{-3}$ M |
| | $10^{-3}$ | – | 0.81 | |
| | $10^{-2}$ | – | 0.15 | |
| | $10^{-5}$ | $10^{-2}$ M glucose | 0.32 | ($3.2 \times 10^{-3}$ M) |

action mixture consisting of D-glucose 14 mg, D-glucose-U-$^{14}C$ 100 $\mu$Ci, Na-ATP 63 mg, $NaHCO_3$ 22 mg, $MgCl_2.6H_2O$ 52 mg, hexokinase 2 mg, in a total volume of 14.2 ml (pH 8.2) was incubated at 37°C for 90 min. One ml HCl (1:1) was then added and the mixture boiled for 1 min. After removing the precipitated protein by centrifugation, glucose-6-phosphate-$^{14}C$ was recovered by the barium-ethanol method [7] which is much more rapid than the ion-exchange chromatography described by the original authors [6].

The labelled glucose-6-phosphate was then equilibrated with a yeast cell membrane fraction (40 min at 30,000 × *g*) as well as with a pure plasmalemma preparation (kindly provided by Prof. Ph.Matile of the ETH, Zürich) which contained no demonstrable hexokinase activity. Results of this binding and of combined binding of glucose and glucose-6-phosphate are shown in table 2. Glucose-6-phosphate, like glucose, is bound to the yeast cell membrane but its binding site appears to be different from the principal binding site of glucose since the dissociation constant of glucose-6-phosphate with the membrane receptor is different from its inhibition constant against glucose binding. Curiously, glucose inhibits the binding of glucose-6-phosphate somewhat more. An important difference appears to lie in the binding capacity of the membrane proteins for glucose and for glucose-6-phosphate (the apparent molarity of binding sites referred to membrane dry weight is about 20 mM for glucose but about 40 mM for glucose-6-phosphate).

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