External K⁺ Affects the Internal Acidification Caused by the Addition of Glucose to Yeast Cells

By EULALIA VALLE, LOURDES BERGILLOS AND SOFIA RAMOS*

Departamento Interfacultativo de Bioquímica, Universidad de Oviedo, 33071 Oviedo, Spain

(Received 19 June 1986)

In glucose-grown cells of *Saccharomyces cerevisiae*, collected during the stationary phase of growth, the addition of K⁺ to the external medium reversed glucose-induced internal acidification in 2 min. However, in ethanol-grown cells external K⁺ did not reverse the effect of glucose even after 20 min. The presence or absence of external K⁺ did not alter the modification of trehalase and fructose-1,6-bisphosphatase induced by glucose. It is concluded that transient acidification may be sufficient to cause the associated transient increase in cAMP.

INTRODUCTION

The addition of glucose to *Saccharomyces cerevisiae* cells grown on a sugar-free medium causes a wide variety of effects by which the yeast adapts its metabolism to the newly supplied carbon source. Some of these effects are mediated by an increase in intracellular cAMP concentration. Thus, the reversible inactivation of fructose-1,6-bisphosphatase (FBPase) (Mazón *et al.*, 1982) and the activation of trehalase and 6-phosphofructo-2-kinase (François *et al.*, 1984) are preceded by an increase in cAMP concentration. Recently, Valle *et al.* (1986) showed that the addition of glucose to ethanol-grown yeasts caused an intracellular acidification, and demonstrated that the trehalase activation caused by the addition of uncouplers and nystatin was mediated by an internal acidification.

Glucose-induced intracellular acidification has been reported previously (den Hollander *et al.*, 1981; Nicolay *et al.*, 1982), and Caspani *et al.* (1985) have recently postulated that the glucose-stimulated increase in cAMP concentration could be mediated by an intracellular acidification. The cause of the decrease of internal pH induced by glucose is not understood, but may be due to the accumulation of either CO₂ (den Hollander *et al.*, 1981) or glucose 6-phosphate (Valle *et al.*, 1986).

In the present work we have examined the effect of external K⁺ on the internal acidification of *S. cerevisiae* in relation to the modification of trehalase and FBPase activities.

METHODS

Materials. Yeast Nitrogen Base was from Difco; [¹⁴C]benzoic acid [29.4 Ci mol⁻¹ (1.088 TBq mol⁻¹)] was from Amersham. All other reagents were from Sigma.

Yeast strain and culture conditions. *Saccharomyces cerevisiae* strain X17/17 (ATCC 52300) (*MAT α his1*) was used. It was cultured at 28 °C with shaking in a medium containing 1.4% (w/v) Yeast Nitrogen Base supplemented with 30 mg histidine l⁻¹ and 2% (w/v) glucose or 2% (v/v) ethanol. Glucose-grown cells were collected 4 to 8 h after the sugar was consumed, at an approximate cell density of 5 × 10⁷ cells ml⁻¹. These cells are referred to as cells at the stationary phase of growth although they had a generation time of 20 h. Ethanol-grown cells were collected during the logarithmic phase, at an approximate cell density of 3 × 10⁷ cells ml⁻¹. In all cases, cells were washed twice with distilled water and resuspended in 40 mM-6-aminohexanoate/HCl buffer, pH 4.5, at the indicated cell density.

Abbreviation: FBPase, fructose-1,6-bisphosphatase.

Fig. 1. Effect of glucose and K^+ on benzoic acid accumulation by *S. cerevisiae* adapted to grow in a sugar-free medium. Glucose-grown cells collected at the stationary phase of growth (a) and ethanol-grown cells collected during the logarithmic phase (b) were resuspended at a concentration of 2×10^8 cells ml^{-1} in 40 mM-6-aminohexanoate/HCl buffer, pH 4.5, in the presence ($\circ$, $\triangle$) or in the absence ($\bullet$, $\blacktriangle$) of 5 mM-KCl. Benzoic acid accumulation and internal pH were determined as described by Valle *et al.* (1986) after addition of $1.4 \mu M$ - $[^{14}C]$ benzoic acid. The arrow indicates the addition of 10 mM-glucose ($\blacktriangle$, $\triangle$); C_i and C_o are the internal and external concentrations, respectively, of $[^{14}C]$ benzoic acid.

pH determination. The internal pH was determined from the accumulation of the weak acid, $[^{14}C]$ benzoic acid. The determinations were done at room temperature and started by the addition of $1.4 \mu M$ - $[^{14}C]$ benzoic acid to the cell suspension. Filtration and radioactivity determination were done as described by Valle *et al.* (1986). The internal pH was determined from the concentration gradient of $[^{14}C]$ benzoic acid, assuming an intracellular volume of $3.4 \mu l$ per 10^8 cells as described by Valle *et al.* (1986).

Enzyme assays. Trehalase activation and FBPase inactivation were determined in yeast homogenates obtained as described by Gancedo & Gancedo (1971), François *et al.* (1984) and Valle *et al.* (1986).

RESULTS

Fig. 1 shows the effect of K^+ and glucose on $[^{14}C]$ benzoic acid accumulation by *S. cerevisiae* grown either on a medium with glucose as carbon source and collected during the stationary phase of growth, or grown on a medium containing ethanol and collected during the logarithmic phase. When stationary phase glucose-grown cells resuspended in K^+ -free medium were used, the accumulation of benzoic acid rapidly reached a steady state at a concentration gradient of 180 (inside with respect to outside), corresponding to an internal pH of 6.9 (Fig. 1a). The concentration gradient was 240 for ethanol-grown cells, from which an internal pH of 7.0 can be calculated (Fig. 1b). The accumulation of benzoic acid was the same in the presence of K^+ in the external medium. In the absence of external K^+ the addition of 10 mM-glucose caused the efflux of the previously accumulated benzoic acid down to a concentration gradient of about 40, both in glucose- and in ethanol-grown cells. Thus in both cases, the internal pH had acidified to a value of 6.3. When 5 mM- K^+ was present externally, the addition of glucose to glucose-grown cells caused less acidification (pH 6.6 1 min after the addition of glucose). This acidification was reversible, and an internal pH near 6.9 was measured from 2 to 4 min after the addition of glucose (Fig. 1a). The reversion was observed only at external K^+ concentrations above 3 mM and its rate was increased by raising the K^+ concentration (not shown). As stated above, the addition of glucose to ethanol-grown cells caused an internal acidification similar to that of glucose-grown cells when K^+ was absent. Concentrations of K^+ up to 50 mM produced a partial inhibition of this acidification (internal pH 6.65 after the addition of glucose), but in contrast to the situation in glucose-grown cells the new value for the internal pH still remained constant 20 min after the addition of glucose; 50 to 60 min elapsed before the internal pH reverted to its initial value (not shown). A similar result in ethanol-grown cells was obtained by increasing the

Fig. 2. Activation of trehalase and inactivation of FBPAse caused by the addition of glucose to glucose-grown cells of *S. cerevisiae* collected at the stationary phase of growth. Cell suspensions (10^8 cells ml⁻¹) in 40 mM-6-aminohexanoate/HCl buffer, pH 4.5, were preincubated for 30 min at 30 °C. Glucose (90 mM) was then added with or without 50 mM-KCl. At the indicated times samples were withdrawn and trehalase and FBPAse activities were assayed in yeast homogenates as described by Gancedo & Gancedo (1971), François *et al.* (1984) and Valle *et al.* (1986). ●, No additions; ○, KCl added; ▲, glucose added; △, glucose and KCl added.

K⁺ concentration to 200 mM (not shown). It is interesting that although an external pH of 4.5 was used in these experiments, a similar effect of K⁺ has been previously observed by using ethanol-grown cells at an external pH of 6.0 (Valle *et al.*, 1986, Fig. 1B).

Most experiments showing increases in cAMP levels (Mazón *et al.*, 1982; Tortora *et al.*, 1983; François *et al.*, 1984) and the subsequent modification of FBPAse (Mazón *et al.*, 1982), trehalase (François *et al.*, 1984) and various other enzymes (Tortora *et al.*, 1983; François *et al.*, 1984; Caspani *et al.*, 1985) have been done with glucose-grown cells collected at the stationary phase and suspended in a medium with K⁺. Thus, it was important to determine whether reversal of glucose-induced acidification was necessary to obtain the cAMP-dependent enzyme modification. Fig. 2 shows that trehalase was activated (Fig. 2a) and FBPAse inactivated (Fig. 2b) after the addition of glucose, independently of the presence or absence of 50 mM-K⁺ in the external medium. This indicates that reversion of the internal acidification is not a necessary prerequisite for the modification of these enzymes.

DISCUSSION

The increase in internal cAMP concentration (Caspani *et al.*, 1985) and the activation of trehalase (Valle *et al.*, 1986) by addition of glucose to cells of *S. cerevisiae* adapted to grow in a sugar-free medium are mediated by an intracellular acidification, the mechanism of which has yet to be elucidated. An accumulation of CO₂ has been suggested, because when a N₂/CO₂ mixture is bubbled through a cell suspension, the glucose-induced internal acidification is not observed (den Hollander *et al.*, 1981). Another possible explanation is that the acidification is due to an accumulation of glucose 6-phosphate (Valle *et al.*, 1986), since the addition of glucose causes a transient increase in the concentration of this metabolite in cells adapted to a glucose-free medium (Tortora *et al.*, 1983; François *et al.*, 1984); this possibility is supported by the fact that 2-deoxy-D-glucose (which is phosphorylated but not subsequently degraded through the glycolytic pathway) causes the same acidification as glucose (Valle *et al.*, 1986), while xylose, which is not phosphorylated inside the cell (Sols *et al.*, 1958), does not. The glucose analogue 3-O-methyl-D-glucose, which can be phosphorylated (Gancedo & Gancedo, 1985; Siverio *et al.*, 1986), does not cause an internal acidification after 20 min treatment (Valle *et al.*, 1986). This lack of effect can be attributed to the low 3-O-methyl-D-glucose phosphorylation activity (100 times lower than for glucose) (Siverio *et al.*, 1986). Accumulation of these metabolites and other organic acids could be the reason for the glucose-induced internal acidification, since one of the known effects of glucose addition is the production and secretion of some of these substances (Duro & Serrano, 1981; Sigler *et al.*, 1981).

The data presented here indicate that the acidification caused by the addition of glucose to glucose-grown cells collected at the stationary phase is transient, lasting only 2 to 4 min, provided that K^+ is present in the external medium (Fig. 1a). Moreover, other studies, in which the internal pH of *S. cerevisiae* was measured by ^{31}P NMR, have also shown the glucose-induced acidification to be transient (den Hollander *et al.*, 1981; Nicolay *et al.*, 1982).

Since K^+ uptake is coupled to H^+ efflux (Peña, 1975; Ramos *et al.*, 1985), the K^+ -induced alkalinization of the cell interior could be due to the electrically coupled activities of the H^+ -ATPase and a K^+ carrier. If so, this mechanism should be regulated differently in glucose-grown cells collected at the stationary phase from that in ethanol-grown cells, in which glucose-induced internal acidification persists even in the presence of 50 mM- K^+ (Fig. 1b).

Finally, the data in Fig. 2 indicate that the modification of trehalase and FBPase takes place both in conditions in which the internal acidification was stable (i.e. in the absence of K^+) and in conditions in which it lasted only briefly (i.e. 2 min in the presence of 50 mM- K^+). This indicates that if an internal acidification mediates the increase in internal cAMP concentration caused by the addition of glucose, transient acidification is sufficient to produce the transient increase in cAMP (Mazón *et al.*, 1982; François *et al.*, 1984; Caspani *et al.*, 1985) which acts as a signal for the activation of the cAMP-dependent protein kinase. Alternatively, H^+ conductors and glucose may not share a common mechanism for increasing the cAMP concentration as suggested by J. M. Thevelein (unpublished).

We wish to thank Drs S. Gascón and F. Barros for helpful discussion and critical reading of the manuscript and Dr Thevelein for allowing us to read his manuscript entitled 'Regulation of the cAMP level in the yeast *Saccharomyces cerevisiae*. II The glucose-induced cAMP signal' before its publication. This work was supported by a grant of the F.I.S.84/1085 and the CAICYT 0856-84.

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