The Mechanism of Intracellular Acidification Induced by Glucose in *Saccharomyces cerevisiae*

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Addition of glucose or fructose to cells of *Saccharomyces cerevisiae* adapted to grow in the absence of glucose induced an acidification of the intracellular medium. This acidification appeared to be due to the phosphorylation of the sugar since: (i) glucose analogues which are not efficiently phosphorylated did not induce internal acidification; (ii) glucose addition did not cause internal acidification in a mutant deficient in all the three sugar-phosphorylating enzymes; (iii) fructose did not affect the intracellular pH in a double mutant having only glucokinase activity; (iv) glucose was as effective as fructose in inducing the internal pH drop in a mutant deficient in phosphoglucose isomerase activity; and (v) in strains deficient in two of the three sugar-phosphorylating activities, there was a good correlation between the specific glucose- or fructose-phosphorylating activity of cell extracts and the sugar-induced internal acidification. In addition, in whole cells any of the three yeast sugar kinases were capable of mediating the internal acidification described. Glucose-induced internal acidification was observed even when yeast cells were suspended in growth medium and in cells suspended in buffer containing K^+ , which supports the possible signalling function of the glucose-induced internal acidification. Evaluation of internal pH by following fluorescence changes of fluorescein-loaded cells indicated that the change in intracellular pH occurred immediately after addition of sugar. The apparent K_m for glucose in this process was 2 mM. Changes in both the internal and external pH were determined and it was found that the internal acidification induced by glucose was followed by a partial alkalization coincident with the initiation of H^+ efflux. This reversal of acidification could be due to the activity of the H^+ -ATPase, since it was inhibited by diethylstilboestrol. Coincidence between internal alkalization and the H^+ efflux was also observed after addition of ethanol.

INTRODUCTION

It has become evident that changes in internal pH (pH_{in}) mediate many important cellular processes (Busa & Nuccitelli, 1984). Intracellular pH is important for the activity of a number of enzymes with pH optima within the physiological range as well as for the efficiency of contractile elements and the conductivity of ion channels (Madshus, 1988). Moreover, pH oscillations seem to be important in controlling the cell cycle and proliferative capacity of cells. Low intracellular pH is common to both prokaryotic and eukaryotic resting cells. This is believed to be one of the reasons for the low metabolic activities of cells in this state (Madshus, 1988). Grinstein *et al.* (1987) reported that in various eukaryotic cells, upon cell activation, the intracellular pH generally rises; however, this alkalization is preceded by a transient internal acidification. The causes of this acidification are not fully understood and differ in various cell types. NADPH oxidation is thought to be important in the acidification observed in cytoplasts activated with phorbol esters (Henderson *et al.*, 1988). In platelets, the internal increase in H^+

Abbreviation: DES, diethylstilboestrol.

concentration caused by platelet-derived growth factor and bombesin is thought to be the result of a rise in intracellular free Ca^{2+} which is exchanged for H^+ (Ives & Daniel, 1987). The further alkalization is mainly due to the activation of the Na^+/H^+ antiport in all mammalian cells. This antiport can be rapidly activated in an ATP-independent fashion by internal acidification or by an ATP-dependent process mediated by a Ca^{2+} -dependent protein kinase or by tyrosine kinases (Grinstein *et al.*, 1987).

In yeasts, it has long been known that the addition of sugars causes the appearance of acids in the external medium. This external acidification, which is due to the activity of a H^+ -ATPase (Goffeau & Slayman, 1981; Serrano, 1984) and to the excretion of acids (Wurst *et al.*, 1980), has been correlated with the internal alkalization observed on addition of a carbon source (Serrano, 1984). However, an exchange of H^+ for K^+ also seems to be involved. This system has been characterized in *Neurospora crassa* (Blaat & Slayman, 1987) and observed in *Saccharomyces cerevisiae* (Valle *et al.*, 1987). This alkalization is preceded by an acidification (den Hollander *et al.*, 1981; Nicolay *et al.*, 1982; Caspani *et al.*, 1985; Valle *et al.*, 1986; Thevelein *et al.*, 1987b) which, in the presence of external K^+ , is transient but in its absence lasts for at least 10 min (Valle *et al.*, 1987). The cause of the decrease of pH_{in} induced by glucose is not fully understood, but may be due to the accumulation of either CO_2 (den Hollander *et al.*, 1981), glucose 6-phosphate (Valle *et al.*, 1987; Eraso *et al.*, 1987) or other organic acids, since one of the known effects of glucose is the production and secretion of these substances (Duro & Serrano, 1981; Wurst *et al.*, 1981).

In *S. cerevisiae* addition of glucose also induces an increase in cyclic AMP (cAMP) levels (van der Plaats & van Soligen, 1974; Mazón *et al.*, 1982). Increased cAMP levels are also observed, in the absence of glucose, when an internal acidification is caused by other means (Caspani *et al.*, 1985; Thevelein *et al.*, 1987a). These results, and the demonstration that adenylate cyclase is twice as active at pH 6.5 as at pH 7.0, lead Purwin *et al.* (1986) to postulate that glucose-dependent increases of cAMP levels are mediated by the fall in pH_{in} . This hypothesis is not generally accepted, since Eraso *et al.* (1987) found that a rise in cAMP precedes the glucose-induced internal acidification. In addition, Thevelein *et al.* (1987b) have shown an increase in cAMP under conditions where acidification was prevented. They therefore considered that internal acidification induced by glucose does not mediate the increase in cAMP in *S. cerevisiae*.

In this paper the time-course of changes in internal and external pH were determined using a fluorescent probe which allowed determination of fast changes in pH_{in} .

METHODS

Yeast strains and culture conditions. The following strains of *Saccharomyces cerevisiae* were used (all obtained from the Yeast Genetic Stock Center, Berkeley, Calif., USA): ATCC 52300 (MAT α his); D 308 (MAT α hxx1 hxx2 adel trp1 his2 met4); P1T8C (MAT α hxx2 glk1 adel); P2T22D (MAT α hxx1 glk1 adel) and D 308.3 (MAT α hxx1 hxx2 glk1 adel trp1 his2 met4). In addition, strain AA28 (MAT α pgi1A25::LEU2 leu2-3-112 ura3-52 trp1) was kindly provided by Dr A. Aguilera (Aguilera, 1986). The yeasts were cultured at 28 °C with shaking. The media used were as follows: YNB [1.4%, w/v, Yeast Nitrogen Base (Difco) and 2%, w/v, glucose or 2%, v/v, ethanol]; YPD (1%, w/v, yeast extract, 2%, w/v, peptone, 2% glucose); YPFruct. (1 yeast extract, 2% peptone, 2%, w/v, fructose and 0.1% glucose). Cells were harvested by centrifugation, washed twice with distilled water and resuspended in the appropriate buffer.

Determination of pH_{in} . Two different methods were used: measuring the accumulation of the weak acid benzoic acid and/or following the changes in fluorescence of fluorescein. Unless indicated the determination of internal pH with [^{14}C]benzoic acid [29.4 Ci mol $^{-1}$ (1.088 TBq mol $^{-1}$; 1.4 μM)] was done using the acid concentration gradient reached at steady state as previously described (Valle *et al.*, 1986), in cells suspended in 50 mM-6-aminohexanoate/HCl buffer, pH 4.5, at a density of 1.6×10^8 cells ml $^{-1}$. The determination of pH_{in} with fluorescein was done according to the method described by Slavik (1982) with some modifications. The cells were washed twice with distilled water and incubated at a concentration of 1.6×10^8 cells ml $^{-1}$ at 30 °C for 20 min in universal buffer (50 mM-6-aminohexanoate, 50 mM-MES, 50 mM-HEPES, adjusted to pH 6.9 with HCl) containing 100 μM -fluorescein diacetate (Sigma). Cells were then washed twice with ice-cold 50 mM-6-aminohexanoate/HCl buffer, pH 4.5, or 50 mM-MES/NaOH buffer, pH 6.0, resuspended in the same buffer at a cell density of 1.6×10^8 cells ml $^{-1}$ and kept on ice until use. For a standard assay, 0.1 ml of this suspension was added to 3 ml 50 mM-6-aminohexanoate buffer, pH 4.5, or 50 mM-MES/NaOH buffer, pH 6.0, and the fluorescence emitted at 520 nm was recorded at 20 °C after excitation at 490 and 435 nm. Fluorescence

measurements were made in a Perkin-Elmer MPF-44A fluorescence spectrophotometer. A 3 ml cuvette was used in which continuous and efficient stirring was ensured by using a small plastic stick coupled to an electric mixer attached to the cover of the sample compartment. The fluorescein fluorescence emitted at $\lambda_{\text{ex. 490 nm}}$ was continuously monitored since it is affected by pH changes whereas the fluorescence emitted at $\lambda_{\text{ex. 435 nm}}$ was recorded for only a few seconds, several times during each experiment. The values of intracellular pH were obtained from the fluorescence ratio 490 nm/435 nm using a calibration curve prepared as described by Slavik (1982).

Potentiometric measurements of pH_{out} . The external H^+ concentration was continuously monitored in a water-jacketted vessel at 20 °C containing 10 ml of cell suspension (0.5×10^8 cells ml^{-1}) in 5 mM-6-aminohexanoate/HCl buffer, pH 4.5. Ingold glass H^+ -sensitive electrodes were used. Electrodes were coupled to a pH meter PHM 64 (Radiometer) and the results were recorded on a two-channel LKB recorder.

Hexose phosphorylating-activity. This was determined in yeast homogenates as described by Fernández *et al.* (1985).

RESULTS

*Effect of sugars on pH_{in} of *S. cerevisiae**

The pH_{in} of cells prior to the addition of the sugars was 6.8. As described previously (den Hollander *et al.*, 1981; Nicolay *et al.*, 1982; Caspani *et al.*, 1985; Valle *et al.*, 1986), the addition of 20 mM-glucose or other sugars which are degraded through glycolysis lowered pH_{in} to about 6.4 (Table 1). Among the non-metabolizable sugars and sugar derivatives tested, those which are not efficiently phosphorylated (Sols *et al.*, 1958; Gancedo & Gancedo, 1985; Siverio *et al.*, 1986) such as xylose (150 mM), 3-*O*-methyl D-glucoside (20 mM) or 6-deoxy-D-glucose (100 mM) had little effect on pH_{in} . However, 2-deoxy-D-glucose (20 mM), which is phosphorylated but not subsequently metabolized, caused a drop of pH_{in} similar to that produced by glucose. 6-Deoxy-D-glucose (100 mM) was used to ensure access of the analogue to the inside of the cells since low-affinity transport systems have been demonstrated whenever glucose or its analogues are transported with no further phosphorylation (Bisson & Fraenkel, 1983; Ongjoco *et al.*, 1987). These results indicate that phosphorylation is required for a sugar to cause intracellular acidification. This hypothesis is further supported by the fact that glucose (100 mM) does not affect the internal pH in strain 308.3 (*hxx1 hxx2 glk*), which lacks the three glucose phosphorylating enzymes (100 mM-glucose was used in this case for the same reasons as explained for 6-deoxy-D-glucose). On the other hand, glucose (20 mM) as well as fructose (20 mM) were equally active in causing internal acidification in strain AA28 (*pgi1Δ*) which lacks phosphoglucose isomerase activity. Addition of sorbitol (150 mM), which is not metabolized by yeasts and had no effect on pH_{in} , was assayed to test for any osmotic effect.

Role of glucose-phosphorylating enzymes on glucose-induced internal acidification

Three phosphorylating enzymes have been described for *S. cerevisiae*: hexokinase PI is a constitutive enzyme (Fernández *et al.*, 1985) with a ratio of fructose to glucose phosphorylating activities of 2.5; glucokinase synthesis is repressed by glucose and is specific for glucose; hexokinase PII is an enzyme whose synthesis is induced by the presence of glucose (Fernández *et al.*, 1985) and which may be involved in catabolite repression (Entian & Mecke, 1981). This latter enzyme catalyses the phosphorylation of fructose and glucose at the same rate (Barnard 1975; Gancedo *et al.*, 1977). To test whether the effect of glucose on pH_{in} was dependent on the activity of any of these enzymes in particular, the intracellular pH of three double mutants (D 308, PIT8C and P2T22D), each having only one of the three phosphorylating enzymes, was determined before and after the addition of 20 mM glucose or fructose. Exponential-phase cells grown on minimal medium with ethanol as carbon source were suspended in 6-aminohexanoate/HCl buffer (50 mM), pH 4.5, and pH_{in} was evaluated from the accumulation of [^{14}C]benzoic acid. Strain D 308 (*hxx1 hxx2*), which has only glucokinase activity, had a pH_{in} of 6.2 in the absence of glucose. In the presence of 20 mM-glucose the pH_{in} was 4.8; addition of fructose (20 mM) had no significant effect. Strain PIT8C (*hxx2 glk*), which has only hexokinase PI, had a basal pH_{in} of 6.6 which fell to 6.4 in the presence of glucose and 5.7 in the presence of fructose. Strain P2T22D (*hxx1 glk*), which has only hexokinase PII, had a basal pH_{in} of 6.4, which fell to about 5.7 in the presence of glucose or fructose.

Table 1. *Effect of different sugars and glucose analogues on pH_{in} of *S. cerevisiae**

pH_{in} was measured in cells suspended in 50 mM-6-aminohexanoate/HCl buffer, pH 4.5, from the distribution at steady state of [^{14}C]benzoic acid (1.4 μ M). Sugars or derivatives were added at time zero. Strain ATCC 52300 was grown in minimal medium with glucose as carbon source and harvested 10 h after the glucose was exhausted in the medium. Strain D 308.3 was grown in minimal medium with ethanol as carbon source and harvested during the exponential phase of growth. Strain AA28 was grown in YPFruct. medium containing 0.1% glucose and harvested during the stationary phase. Values are means of different experiments (number given in parentheses).

Strain	Addition	pH_{in}
ATCC 52300	None	6.85 (28)
	Glucose (20 mM)	6.41 (12)
	Fructose (20 mM)	6.36 (4)
	2-Deoxy-D-glucose (20 mM)	6.45 (6)
	6-Deoxy-D-glucose (100 mM)	6.80 (5)
	3-O-Methylglucoside (20 mM)	7.00 (4)
	Xylose (150 mM)	7.05 (2)
	Sorbitol (150 mM)	6.89 (3)
D 308.3 (<i>hxx1 hxx2 glk</i>)	None	6.93 (3)
	Glucose (100 mM)	6.92 (3)
AA28 (<i>pgi1</i> Δ)	None	6.52 (3)
	Glucose (20 mM)	5.90 (3)
	Fructose (20 mM)	5.85 (3)

Table 2. *Relationship between glucose- and fructose-phosphorylating activities and the change in pH_{in} induced by addition of glucose or fructose to mutants having only one of the three sugar kinases*

Strains were grown and harvested as indicated in the legend to Fig. 1. The sugar-phosphorylating specific activities were determined in cell-free extracts; Values are means of two different experiments. The values for decrease of pH_{in} (ΔpH) induced by addition of glucose or fructose are taken from data described in the text and are means of five independent determinations.

Strain (enzyme present)	Specific activity [μ mol min $^{-1}$ (mg protein) $^{-1}$]		ΔpH	
	Glucose	Fructose	Glucose	Fructose
D 308 (glucokinase)	917	168	-1.44	-0.06*
P1T8C (hexokinase PI)	207	610	-0.24	-0.90
P2T22D (hexokinase PII)	561	561	-0.67	-0.53

* Not statistically significant.

The specific activity of glucose- and fructose-phosphorylating enzymes of these strains was measured in extracts of cells collected under the same conditions as above, in order to correlate these values with the effect of the sugars on the pH_{in} of whole cells. A good correlation was observed between these two parameters (Table 2). Thus strain D 308, where glucose induced the largest acidification, also had the highest specific glucose-phosphorylating activity. Furthermore fructose, which showed no significant effect on pH_{in} in this strain, had the lowest specific fructose-phosphorylating activity. The correlation between hexose-phosphorylating specific activities and intracellular acidification also held for strains P1T8C and P2T22D. These results are further evidence that the acidification induced by the addition of glucose is due to the phosphorylation of the sugar and that any of the three sugar kinases are able to mediate this effect.

Effect of glucose addition to cells suspended in growth medium

The above data show that glucose causes a drop of pH_{in} when cells are resuspended in 50 mM-6-aminohexanoate/HCl buffer, pH 4.5. However, it has been shown that in the presence of

Fig. 1. Effect of glucose addition on pH_{in} of cells suspended in growth medium. *S. cerevisiae* ATCC 52300 was grown to stationary phase in minimal medium with glucose as carbon source at 28 °C with shaking. A culture sample (3 ml) containing 5×10^7 cells ml^{-1} was transferred to a 25 ml flask and further incubated for 2 h with shaking. Then [^{14}C]benzoic acid (4.25 μM) was added and samples subsequently analysed as described in Methods. The acid distribution and pH_{in} were determined taking into account the external pH (3.1) of the medium. ○, Control; △, 10 mM-glucose (the arrow indicates the time of addition). Similar results were obtained in three different determinations.

external K^+ the acidification induced by glucose is transient and is followed by an alkalization (Valle *et al.*, 1987). If the acidification caused by glucose has any signalling function it should also occur in cells suspended in culture medium. This is precisely the case shown in Fig. 1, where pH_{in} was evaluated from [^{14}C]benzoic acid accumulation from the same medium in which the cells were growing. The pH_{in} of cells in these conditions was 7.1. Addition of glucose caused a sudden drop which varied between 0.1–0.3 pH units in different experiments, followed by a rise of pH_{in} to a value of 7.7, 6 min after addition of glucose.

Time-course of internal and external acidification after addition of a carbon source

The change in pH_{in} represents a balance between H^+ efflux and H^+ formation due to the phosphorylation of glucose. The time-course of these two processes was studied when glucose was added to cells in the stationary phase of growth. The external pH (pH_{out}) was determined with pH electrodes and the changes in pH_{in} were evaluated in fluorescein-loaded cells. This pH-dependent fluorescent dye was used because it allows better time resolution of pH changes. Fig. 2a shows that before addition of glucose, both pH_{out} (trace A) and pH_{in} (traces B and C) were steady. Addition of 10 mM-glucose caused an immediate decrease in the fluorescein fluorescence ratio 490 nm/435 nm. This represents an intracellular acidification which had a maximum of 0.4 pH units and was reached 30 s after the addition of sugar. A partial internal alkalization followed which, in the absence of external K^+ , remained below the original value; in the presence of 10 mM external K^+ , pH_{in} returned to values higher than the original (see below). pH_{out} was also lowered after addition of glucose, but only after a 30 s delay. Thus delay in H^+ efflux was coincident with the start of intracellular alkalization, suggesting that the change in pH_{in} may represent the activity of the H^+ -ATPase. This is supported by the effect of the H^+ -ATPase inhibitor diethylstilboestrol (DES). In its presence the change in cell fluorescence which followed the acidification induced by glucose was not observed (Fig. 2a, trace C). Contrary to what has been described by Serrano (1980) in starved cells, we were unable to observe any effect of DES on external acidification when freshly harvested cells were treated with this inhibitor. The lack of effect of DES on H^+ efflux might indicate that the contribution of the membrane-bound H^+ -ATPase to the external acidification under these conditions is small and that the change in pH_{in} determined with fluorescein could be a more reliable method to assay *in vivo* the activity of the H^+ -ATPase than the acidification of the external medium, in which acid efflux may also be involved (Duro & Serrano, 1981; Wurst *et al.*, 1980).

Simultaneous H^+ efflux and internal alkalization were also observed (Fig. 2b) when the effect of ethanol and H_2O_2 on the fluorescence of fluorescein-loaded cells (trace B) and H^+ efflux

Fig. 2. Time-course of external and internal acidification after the addition of glucose (a) and ethanol (b) to *S. cerevisiae*. (a) Cells of *S. cerevisiae* ATCC 52300, grown and harvested as in Table 1, were suspended (0.5×10^8 cells ml^{-1}) in 5 mM-6-aminohexanoate/HCl buffer, pH 4.5. A sample (2.5 ml) of this suspension was transferred to a cuvette with stirring and the external pH monitored with a pH electrode (trace A). The arrow indicates addition of 10 mM-glucose (Glc). For measurement of pH_{in} (traces B and C) the cells were loaded with fluorescein and suspended (1.6×10^8 cells ml^{-1}) in 50 mM-6-aminohexanoate/HCl buffer, pH 4.5. A sample (100 μl) of this suspension was added to 3 ml of the same buffer in a spectrofluorimeter cuvette. Traces B and C show the intracellular pH changes calculated from the ratio of fluorescence emitted at 520 nm when the excitation wavelength was 490 nm or 435 nm (see Methods). When indicated, 10 mM-glucose or 16 μg DES ml^{-1} was added. (b) (trace A). The external pH was determined as described above (in trace A) but with cells grown in minimal medium with 2% (v/v) ethanol as carbon source and harvested in the late exponential phase of growth. When indicated, ethanol (EtOH) (0.1%) and H_2O_2 (0.005%) were added. For trace B, pH_{in} was determined by the fluorescence method but in cells grown on ethanol as carbon source.

(trace A) were determined. H^+ efflux was observed without any delay after the addition of the carbon source, which agrees with the fact that the alkalization of the internal medium was not preceded by an acidification (trace B). The addition of glucose to these ethanol-grown cells caused effects similar to those described for glucose-grown cells in Fig. 2(a) (data not shown).

Effect of external K^+ on glucose-induced internal acidification

External K^+ , in the presence of glucose, caused an intracellular alkalization (Valle *et al.*, 1987). However, Eraso *et al.* (1987) found that at an external pH of 6.0, and in the presence of K^+ , addition of glucose did not produce an appreciable internal acidification as measured with weak acids. Since the change in pH_{in} induced by glucose in cells suspended in culture medium (Fig. 1) was smaller and more variable than that determined in cells suspended in buffer (described above) it was important to determine the extent of the glucose-induced internal acidification in the presence of K^+ by the fluorescein method. Addition of 10 mM-glucose caused a transient decrease in fluorescein fluorescence, corresponding to a fall of 0.5–0.6 pH units, at pH_{out} values of 4.5 or 6.0 (Fig. 3). This decrease is similar to the values observed in the absence of external K^+ (Fig. 2a, trace B). Acidification was followed by a greater intracellular alkalization than observed in the absence of K^+ , pH_{in} reaching values higher than the original.

Kinetics of glucose-induced internal acidification

The rate of decrease of fluorescence after addition of glucose depended on the concentration of added glucose (Fig. 4). The apparent K_m for glucose-induced acidification was 2 mM, a value

Fig. 3. Effect of external K^+ on glucose-induced internal acidification in *S. cerevisiae*. Fluorescein-loaded cells were suspended in 50 mM-6-aminohexanoate/HCl buffer, pH 4.5, plus 10 mM-KCl (a) or in 50 mM-MES/NaOH buffer, pH 6.5, plus 10 mM-KCl (b). The fluorescence emitted at 520 nm when the excitation wavelength was 490 nm or 435 nm is shown. Values of pH_{in} determined from the fluorescence ratio 490 nm/435 nm are given.

Fig. 4. Dependence of the rate of internal acidification on the concentration of added glucose. Cells were harvested, loaded with fluorescein and suspended in 50 mM-6-aminohexanoate/HCl buffer, pH 4.5. Fluorescence was recorded as in Fig. 3. The arrow indicates the addition of the concentrations of glucose indicated. Inset, Eadie-Hofstee plot of rates of decrease in fluorescence and the glucose concentrations shown, where v is represented in relative fluorescence units min^{-1} and $[\text{glucose}]$ in mM.

similar to that of the high-affinity glucose carrier which has a K_m of 1.5 mM (Bisson & Fraenkel, 1983) and higher than the K_m values of kinases, which are below 1 mM (Purich *et al.*, 1973). This is not in conflict with data reported above where intracellular acidification induced by glucose was reported to be dependent on the phosphorylation of the sugar, because glucose transport is rate-limiting for sugar phosphorylation and hence for the acidification caused by this reaction. However, the K_m value determined for glucose for the glucose-induced internal acidification is

very different from the K_m (25 mM; Beullens *et al.*, 1988) for glucose of the 'triggering reaction' that causes the increase of internal cAMP levels.

DISCUSSION

The results presented in this paper clearly show that the acidification induced in *S. cerevisiae* by the addition of glucose and other sugars, which are metabolized via the glycolytic pathway, is due to the phosphorylation of the sugar. This is supported by the lack of acidification observed when glucose was added to a mutant (D 308.3) deficient in all three sugar phosphorylating enzymes. The same lack of acidification was observed when sugars which are not efficiently phosphorylated were added. Moreover, since addition of glucose to the mutant AA28 (*pgi* Δ), which is unable to metabolize glucose 6-phosphate, caused an acidification similar to that observed in the wild-type, it can be concluded that the initial triggering reaction for glucose-induced intracellular acidification is located after the transport process and before glucose 6-phosphate isomerization. Any of the three glucose-phosphorylating enzymes in yeasts can mediate acidification upon glucose addition. The acidification could be due to the H^+ ion which is released during phosphorylation, since glucose 6-phosphate has a pK of 1.4 while the pK of dissociable groups of ATP is near 7.0. This excludes the possibility that CO_2 or any other organic acids derived from glucose metabolism could be implicated in acidification since glucose, which is not metabolized beyond glucose 6-phosphate in the *pgi* Δ mutant, affects pH_{in} . In addition, the possibility of H^+ generation through the oxidation of glucose by the pentose phosphate shunt can be discounted since in strain AA28 fructose is as effective as glucose at inducing acidification. This strain requires small amounts of glucose to grow in a medium with fructose as carbon source (Aguilera, 1986), since glucans (Ballou, 1982) and inositol (Henry, 1982), as well as NADPH, are generated in yeasts from glucose 6-phosphate through direct oxidation (see Fraenkel, 1982). The glucose requirement of this strain indicates that fructose 6-phosphate cannot enter the pentose phosphate shunt.

The acidification caused by glucose was also observed in cells suspended in culture medium. In this case, the acidification was transient, as expected, due to the presence of K^+ in the culture medium (Valle *et al.*, 1987). Interestingly, pH_{in} was 7.1 even though the pH of the medium was 3.1. This result conflicts with those of Borst-Pauwels (1981) and de la Peña *et al.* (1982), who showed that pH_{in} was a function of pH_{out} in yeasts suspended in buffer with or without glucose. Our results indicate that when yeasts are suspended in culture medium, even if the sugar has been exhausted, they are more effective at maintaining their pH_{in} than when suspended in buffer.

It is well-accepted that the internal acidification produced by H^+ conductors causes an increase in cAMP levels (Mazón *et al.*, 1982; Thevelein *et al.*, 1987a) and that the effect of uncouplers requires at least one functional *RAS* gene, a fact indicating its physiological importance (Mbonyi *et al.*, 1988). However, the question whether glucose-induced acidification mediates the increase in cAMP levels remains unanswered.

The results presented in this paper indicate that the internal acidification induced by glucose addition is a function of the concentration of glucose added but with a value for the apparent K_m of 2 mM. The different values for the glucose-triggered increase in cAMP levels and internal acidification might indicate that in addition to the internal acidification the signal that glucose produces is dependent on a different system with a lower affinity. It might well be the binding to a receptor [Beullens *et al.* (1988) support the hypothesis of the low-affinity transport system bound to a kinase], which, as demonstrated by these authors, is only expressed in glucose-derepressed cells. In conclusion, our results indicate that although internal acidification might mediate the glucose-induced increase in cAMP, another mechanism with a lower affinity might also be involved. How the possible receptor interacts with adenylate cyclase and the GTP-binding RAS protein is not known at present.

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