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Activation of yeast plasma membrane ATPase by acid pH during growth

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When yeast grows on media in which a final external pH lower than 4 is attained there is a 2–3-fold increase in plasma membrane ATPase activity. This acid-mediated activation produces a 2-fold increase in the ATPase affinity for ATP but does not modify its optimum pH. The acid-mediated activation is dependent on the stage of growth, only late logarithmic or stationary cells being capable of being activated by incubation in an acidic buffer. Only cells able to activate the ATPase maintain a constant internal pH when incubated in acidic buffers. It is concluded that acid-mediated activation of the plasma membrane ATPase is a mechanism of maintaining constant internal pH during growth of yeast on acid media.

ATPase; pH control; (Yeast)

1. INTRODUCTION

Yeast produces an acidification of the external medium during growth on glucose and other sugars. This external acidification is closely associated with the metabolism of the sugar [1] and its magnitude depends on the buffering capacity of the growth medium. In some cases the initial pH of the culture decreases by 2 or 3 pH units and pH values as low as 3.5 can be attained when the stationary phase of growth is reached [2]. In media with a pH value below that of the cytoplasm the passive movement of protons across the plasma membrane would tend to acidify the cytoplasm. Nevertheless, yeast cells are able to maintain their internal pH between 6 and 7.5 when the extracellular pH varies from 3.5 to 9 [2–4]. This is mainly due to the contribution of plasma membrane ATPase activity which seems to be implicated in internal pH regulation [5].

We have reported some preliminary results showing an increase of plasma membrane ATPase activity during growth of yeast on a medium in which a great external acidification is produced [6]. The purpose of the present work was to investigate whether this observed ATPase increase is due to the medium acidification and to determine how this is related to the maintenance of the internal pH. The results indicate that during growth of yeast on acid media there is an activation of the plasma membrane ATPase and that this is a mechanism for regulating internal pH.

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Abbreviations: YPD, yeast extract, peptone, dextrose; MM, mineral medium

2. MATERIALS AND METHODS

2.1. *Growth conditions*

Saccharomyces cerevisiae X2180 was grown at 30°C either on a complex medium with 1% yeast extract and 2% peptone (YPD) or on a mineral medium (MM) [7] using NaCl (0.25 g/l) instead of

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the original sodium citrate. Where indicated, urea (3 g/l) (MM-urea) or proline (1 g/l) (MM-Pro) were used instead of NH_4^+. The carbon source was in all cases 2% glucose. The initial pH of the MM was adjusted to 5.5. In some cases the MM was buffered with 0.1 M Mes (pH 6). The media were inoculated with yeast grown to stationary phase on a medium of the same composition as that to be inoculated. Growth was followed by measuring the turbidity of the culture at 660 nm.

2.2. *Sampling of yeast*

Culture aliquots (50–70 mg dry wt) were collected by centrifugation, washed twice with deionized water, resuspended in 4 ml of 0.1 M Mes adjusted to pH 6.5 with Tris and incubated with agitation for 30 min at 30°C to eliminate glucose activation of the plasma membrane ATPase [8]. Then, a concentrated solution of Tris, EDTA and dithioerythritol to give final concentrations of 25, 5 and 2 mM, respectively, was added and the samples frozen in liquid nitrogen and stored at −70°C until use.

2.3. *Conditions of ATPase activation*

Yeast cells were harvested by centrifugation, washed twice with deionized water and resuspended at 13–18 mg dry wt/ml in 0.1 M Mes adjusted to pH 6.5 with Tris. After 30 min of incubation at 30°C samples of 4 ml were taken (time 0). Then, 0.1 M glucose was added to the rest of the suspension for glucose activation or it was centrifuged, washed once with deionized water and resuspended at the same cell concentration in 50 mM succinic acid adjusted to pH 3.5 with Tris for acid activation. Samples of 4 ml were taken after 10 min for glucose activation or after 30 min for acid activation when no other times are indicated. Samples were frozen as above except that Tris was 100 mM instead of 25 mM for samples in buffer of pH 3.5.

2.4. *Homogenization and membrane preparation*

Frozen samples were thawed and homogenized by vigorous vortex-mixing with 5 g glass beads (0.5 mm diameter) for 5 min. Total membrane fraction and purified plasma membranes were prepared as in [8].

2.5. *ATPase assay*

Plasma membrane ATPase from total membranes (20–60 μg) was assayed as in [8] except that lysolecithin was omitted. Assay of the enzyme in purified preparations (3–15 μg) was according to [8] but the purified plasma membranes (0.2 mg/ml) were incubated for 2–3 min at 30°C with lysolecithin (0.2 mg/ml) prior to assay with sonicated soya phospholipids (0.4 mg/ml). Unless otherwise indicated, assays were performed at pH 6.5 with 2 mM ATP.

2.6. *Protein determination*

Protein concentration was determined by a modified Bradford method [9] with bovine serum albumin as standard.

2.7. *Determination of internal pH*

Yeast cells were harvested by centrifugation, washed twice with deionized water and resuspended at 4 mg dry wt/ml in 0.1 M Mes adjusted to pH 6.5 with Tris. After 30 min incubation, the yeast suspension was centrifuged, washed once with water and resuspended in the buffer indicated in the figure legends. Internal pH was measured with labelled benzoic acid as in [10].

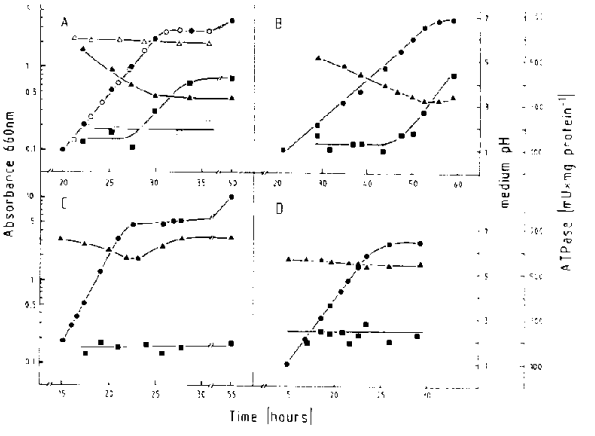

Fig.1. ATPase activity during yeast growth on different media. Yeast was grown on: (A) MM (filled symbols) and MM buffered with 0.1 M Mes, pH 6 (empty symbols), (B) MM-Pro, (C) YPD, (D) MM-urea, as described in section 2. Growth was followed by measuring the absorbance at 660 nm and samples were taken for the determination of pH in the medium and plasma membrane ATPase activity. (A–D) (●—●) Absorbance at 660 nm, (▲—▲) pH of the medium, (■—■) plasma membrane ATPase activity.

3. RESULTS

3.1. *ATPase activity increase associated with growth medium acidification*

Plasma membrane activity was measured in yeast during growth on several media in which a different extent of external acidification had been attained.

During growth of yeast on MM (fig.1A) or MM-Pro (fig.1B) an external pH lower than 3.5 was reached and in these cases plasma membrane ATPase activity increased 2–3-fold. On the other hand, when the external pH did not decrease markedly as in MM buffered with Mes, pH 6 (fig.1A), YPD (fig.1C) or MM-urea (fig.1D), wherein the pH is never lower than 5.5, the ATPase activity did not change significantly.

3.2. *ATPase activation by external acidification*

One possibility to explain the increase of ATPase activity observed would be an increase in the quantity of ATPase protein during yeast growth on acid media. However, measurements of the amount of ATPase protein with a quantitative dot-immunoblotting assay failed to show a significant increase in the amount of protein ([11] and unpublished). Another possibility would be an activation of the enzyme. To check this, yeast was grown to nearly stationary phase in YPD or MM buffered with Mes, pH 6, wherein ATPase activity is maintained at a low and constant level (fig.1C,A), and incubated in an acidic buffer. In

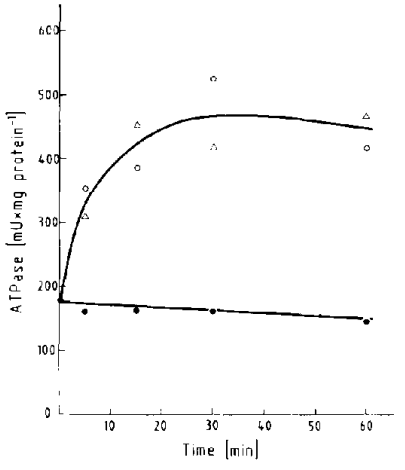

Fig.2. Activation of yeast plasma membrane ATPase by external acidification. Yeast was grown on YPD to an absorbance at 660 nm of 3–4. Different aliquots of the culture were activated as described in section 2. (●—●) 0.1 M Mes-Tris, pH 6.5; (○—○) 50 mM succinic acid-Tris, pH 3.5; (△—△) 50 mM succinic acid-Tris, pH 3.5 + 10 μg/ml cycloheximide.

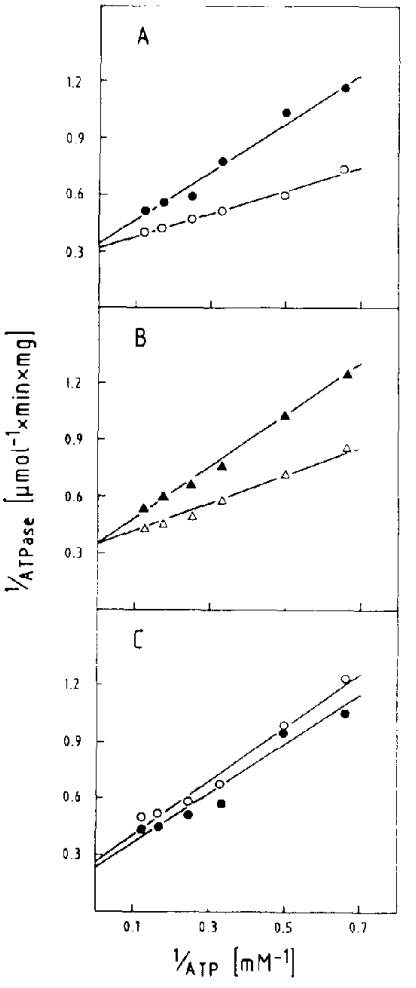

Fig.3. Effect of acidification on the apparent affinity constant of ATPase for ATP. Yeast was grown on (A) MM or (C) MM buffered with 0.1 M Mes, pH 6, collected in early logarithmic (●—●) or stationary (○—○) phase of growth and treated as described in section 2.2. (B) Yeast was grown and treated as in fig.2. (▲—▲) 0.1 M Mes-Tris, pH 6.5; (△—△) 50 mM succinic acid-Tris, pH 3.5. Purified plasma membranes were obtained (see section 2) and ATPase activity was assayed at pH 5.7 at different ATP concentrations. The lines were fitted by using a standard linear regression program (p = 0.97–0.99).

fig.2 the results with YPD grown cells are shown. When yeast is resuspended in a pH 3.5 buffer there is a rapid activation of the ATPase with a maximum increase of nearly 3-fold being reached at 30 min. The same result was obtained in the presence of cycloheximide to inhibit protein synthesis.

3.3. *Modification of kinetic parameters by acid-mediated activation*

We measured the K_m for ATP and the optimum pH in purified plasma membranes of yeast under different conditions.

In logarithmic cells grown in MM the ATPase had a K_m for ATP of 4 mM, the value decreasing to 2 mM when these cells reached the stationary phase (fig.3A) (see ATPase activity under the same conditions in fig.1A). The same effect on the K_m was observed when the ATPase was activated by acidification with an acidic buffer. In this case the K_m for ATP also decreased from 4 to 2 mM after 30 min of incubation in a pH 3.5 buffer (fig.3B) (see ATPase activity under these conditions in fig.2). On the other hand, when yeast was grown in MM buffered with Mes, pH 6, wherein there is no ATPase activity increase when the stationary phase is reached (fig.1A), there was no change in the ATPase K_m for ATP (fig.3C).

Under all these conditions the optimum pH did not vary, being 5.6–5.7 (not shown).

3.4. *Dependence of acid-mediated activation on the phase of growth*

Yeast plasma membrane ATPase is activated by glucose [8]. This glucose-mediated activation is higher in logarithmic cells growing on glucose than in the stationary cells (Serrano, R. and Sánchez, J., personal communication). To investigate whether the activation produced by acidification could be mediated by the same activation system as for glucose, glucose and acid activations were compared at different stages of the phase of growth.

As shown in table 1, ATPase was not activated by incubation in an acidic buffer when yeast was grown on MM and harvested at the logarithmic phase of growth. When collected in the stationary phase of growth in MM, the ATPase was already activated because of the growth medium acidification and the ATPase activity did not change with further incubation at pH 3.5. When yeast was grown on MM buffered to pH 6, the ATPase activity did not vary during growth and only when the yeast cells were harvested in late logarithmic or stationary phase was the ATPase activated when incubated in an acidic buffer. Nevertheless, activation of the ATPase by glucose was produced in

Table 1

Activation of plasma membrane ATPase by acid or glucose at different stages of growth

Growth medium	Growth phase (absorbance at 660 nm)	Medium pH	ATPase activity (mU·mg protein^{-1})			
			pH 3.5		Glucose	
			−	+	−	+
MM	early log (0.22)	5.5	148	131	−	−
	stationary (2.8)	2.8	422	425	−	−
Buffered MM	early log (0.23)	6	160	203	200	1112
	late log (1.5)	5.8	127	257	−	−
	stationary (2.9)	5.7	192	392	169	1313
YPD	log (0.54)	6.3	−	−	138	1073
	stationary (5.3)	6.2	−	−	162	990
	stationary (10.8)	6.6	−	−	190	294

Yeast was grown on MM, MM buffered to pH 6 with 0.1 M Mes or YPD and harvested at different stages of growth. Plasma membrane ATPase was activated by incubation with 50 mM succinic acid-Tris buffer, pH 3.5, or 0.1 M glucose as described in section 2

logarithmic and stationary cells grown on MM buffered to pH 6 or YPD and decreased when yeast was growing on the ethanol produced during glucose fermentation (YPD, stationary 10.8).

3.5. *Cells with increased ATPase activity maintain high internal pH in acid media*

We next examined whether cells with increased ATPase activity had a higher capacity to maintain constant internal pH than cells with low ATPase activity when submitted to acid media.

Yeast was grown on MM and harvested during the early logarithmic or stationary phase of growth and transferred to different buffers with pH ranging from 6 to 3.5. Only yeast cells with increased ATPase activity (stationary) could maintain a constant internal pH and this amounted to only 0.1 pH units lower at pH 3.5 than at pH 6 (fig.4A). Nevertheless, cells with low ATPase activity that cannot be activated by acidification (logarithmic), only maintain a constant internal pH at external pH above 4, although this is lower than the external pH decrease (fig.4B).

When cells had low ATPase activity which was capable of being activated by acidification (stationary cells grown on MM buffered with Mes, pH 6) the same results as those depicted in fig.4A were obtained.

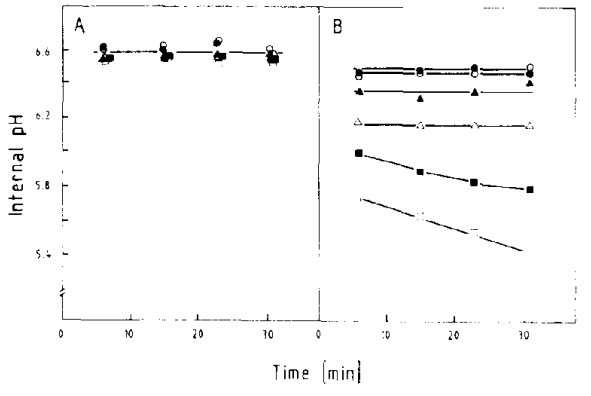

Fig.4. Changes in internal pH as a function of the pH of the medium in cells with high or low ATPase activity. Yeast was grown on MM, collected in either the stationary (A) or logarithmic (B) phase of growth and the internal pH was determined as indicated in section 2 when resuspended in 50 mM succinic acid adjusted with Tris to pH 6 (●—●), 5.5 (○—○), 5 (▲—▲), 4.5 (△—△), 4 (■—■), or 3.5 (□—□).

4. DISCUSSION

The results described here show that acidification of the external medium during yeast growth causes a 2–3-fold activation of the plasma membrane ATPase. There is physiological evidence for the regulation of the yeast plasma membrane ATPase by a variety of factors including intracellular pH [12], extracellular pH, glucose and ATP content of the cells [13], but only sugars have been shown to produce an activated state of the ATPase [8,14]. The acid-mediated activation of the ATPase differs from that with glucose in several respects. Glucose increases 4-fold the affinity of the enzyme for the substrate and modifies the optimum pH for the enzymatic activity to a more alkaline value, but these changes are reversible [8]. Acid-mediated activation produces a 2-fold increase of the affinity for ATP but does not change the optimum pH and the activation remains after incubation at pH 6.5 for 30 min after the acid activation has been produced. In fact, all the samples, as indicated in section 2, were incubated for 30 min at pH 6.5 to eliminate glucose activation. When glucose activation was not eliminated the same increase of ATPase activity during growth on media in which an acidification is produced was obtained but the specific activity was higher (not shown). This fact points out that both activations are additive. Another difference between both activations is that acid activation is only produced in late logarithmic or stationary cells while glucose activation is also produced in logarithmic cells and in this case the activation is even higher than in the stationary cells.

The mechanism of activation by acidification has not been elucidated. We can exclude synthesis of proteins since cycloheximide did not prevent the activation by acid pH and also because there is no increase of ATPase protein as determined using antibodies. The mechanism of glucose activation also remains unclarified [5]. The proposal of cAMP-dependent phosphorylation has been put forward [8] but it has recently been shown that the ATPase is also capable of being activated through a cAMP-independent process [15]. Acid-mediated activation could represent an additional regulatory mechanism of this essential enzyme in yeast.

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