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Half-life of the plasma membrane ATPase and its activating system in resting yeast cells

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The stability of the yeast plasma membrane ATPase and its activating system has been investigated in resting *Saccharomyces cerevisiae*. The half-life of ATPase in the presence of glucose is about 11 h whereas in the presence of ethanol it is > 30 h. In the case of the ATPase activating system half-life values of about 5 and 14 h have been observed, respectively, in the presence of these substrates. These results indicate that, similarly to sugar transport systems, plasma membrane ATPase as well as its activating system are less stable than the bulk of proteins in this organism. The fact that all plasma membrane proteins so far examined show low half-life values suggests that a low stability could be a general characteristic of these proteins.

The bulk of proteins in *Saccharomyces cerevisiae* is quite stable showing half-life values > 70 h [1,2]. However, there is a small group of proteins in this organism, about 5% of the total, that behave differently showing half-lives of about 3 h [2]. Sugar transport systems apparently belong to this later group. These transports are irreversibly inactivated in yeast cells when protein synthesis is inhibited and this inactivation follows first order kinetics and is an energy-dependent process stimulated by fermentable substrates [3–7]. These facts suggest that inactivation of the sugar transports is due to proteolysis of these proteins. To see whether a low stability is a peculiarity of the sugar transports or also affects other plasma membrane proteins we have investigated the stability of the plasma membrane ATPase and of its activating system.

Yeast plasma membrane ATPase is an extensively studied enzyme implicated in the maintenance of the intracellular pH and in active transport of nutrients [8–10]. A still unidentified system activates *in vivo* the plasma membrane ATPase by increasing its optimum pH value and its affinity for ATP [11]. This system is triggered by fermentation [11,12]. We have investigated the stability of ATPase and of its activating system by following the decrease of their activity upon inhibition of protein synthesis in exponentially growing cells. In the case of ATPase, the decrease in the content of this protein has also been measured by using an immunobinding assay. The results show that ATPase as well as its activating system are quite unstable as compared with the bulk of proteins of this organism.

^{125}I-labelled protein A, and [^{35}S]methionine were from Amersham International (Amersham, U.K.). Cycloheximide, phenylmethylsulfonyl fluoride, diethylstilbestrol and ATP were from Sigma Chemical Co. (St Louis, MO). All other reagents were of analytical grade. Strain ATCC 42407 was grown aerobically with 2% glucose in minimal medium as described in Ref. 13. Cell growth was monitored by optical absorbance measurement at 640 nm or by dry weight determination. Conditions for ammonium starvation were as in Ref. 6. Crude extracts were obtained from the cellular homogenate after centrifugation at 3000 rpm as described in Ref. 14. Crude and purified plasma membrane preparations were obtained from washed cells (non-fermenting cells) by differential and sucrose gradient centrifugation as previously described [14]. When indicated the cells were treated with glucose before homogenization (fermenting cells) as in Ref. 11. ATPase activity was measured as in Ref. 14. Protein was determined after precipitation with 5% trichloroacetic acid by the method of Lowry et al. [15]. To measure the content of ATPase in resting cells the immunoassay described in Ref. 16 was used. Duplicate samples containing, respectively, 0.1, 0.2, 0.5 and 1 mg protein, were diluted with 400 ml of a mixture of 10 mM Tris-HCl and 0.15 M NaCl (pH 7.4) and

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applied to nitrocellulose filters with a Manifold device (Bethesda Research Laboratories) suited for quantitative densitometry. Filters were processed as described in Ref. 16 employing an 1/1000 dilution of anti-ATPase antiserum [17] and ^{125}I-protein A. After autoradiography for about 15 h at −70°C with an intensifying screen, the developed film was scanned and, to quantify the results, the height of the peaks was measured. A linear response was observed in the range of protein used.

To measure the half-life of ATPase in glucose growing cells pulse and chase experiments were performed. About 170 mg of cells (dry weight) were harvested from exponentially growing cultures and diluted in 6 ml of fresh medium containing 170 μCi/ml [^{35}S]methionine. After incubation at 30°C for 90 min, cells were harvested, washed, and suspended in 2 ml of 10 mM methionine. After incubation at 30°C for 20 min, cells were suspended in fresh medium to a cellular density of about 8 μg/ml and incubated at 30°C for 12 h. Then, plasma membrane was purified [14] and, after running the samples in polyacrylamide gel electrophoresis as described in Ref. 18, the radioactivity present in the ATPase band was counted.

The half-life of the plasma membrane ATPase in resting yeast was investigated after addition of cycloheximide or deprivation of a nitrogen source to glucose growing cells. The inhibition of protein synthesis by both procedures produced a decrease in the ATPase content of the plasma membrane that followed first order kinetics (Fig. 1A) indicating a half-life for this protein of about 11 h. When, instead of glucose, ethanol was present as energy source addition of cycloheximide was without a detectable effect (Fig. 1A) and the calculated half-life of this enzyme was > 30 h.

In the case of glucose growing yeast, pulse and chase experiments showed that the decay in the radioactivity of the plasma membrane ATPase band was the one expected taking into account the protein dilution due to the cellular growth (results not shown). In other words, a decay due to degradation of this protein was not detected indicating that the half-life of this enzyme in growing cells is > 20 h, that is greater than in resting cells.

The loss in ATPase content was accompanied by a decrease in its activity (Fig. 1B). However, a discrepancy between both parameters was observed that was dependent on the carbon source present in the medium. In the presence of glucose, the activity of ATPase decreased at lower rate ($T_{\frac{1}{2}} = 17$ h) than the content of this enzyme ($T_{\frac{1}{2}} = 11$ h). A similar discrepancy between activity and antigenicity has been previously reported in mutants with reduced expression of ATPase that has been explained by an activation of the enzyme [19,20]. Indeed activation processes of the yeast plasma membrane ATPase have been shown to occur *in vivo* [11,21] giving plausibility to this explanation. On the other hand, our results obtained in the

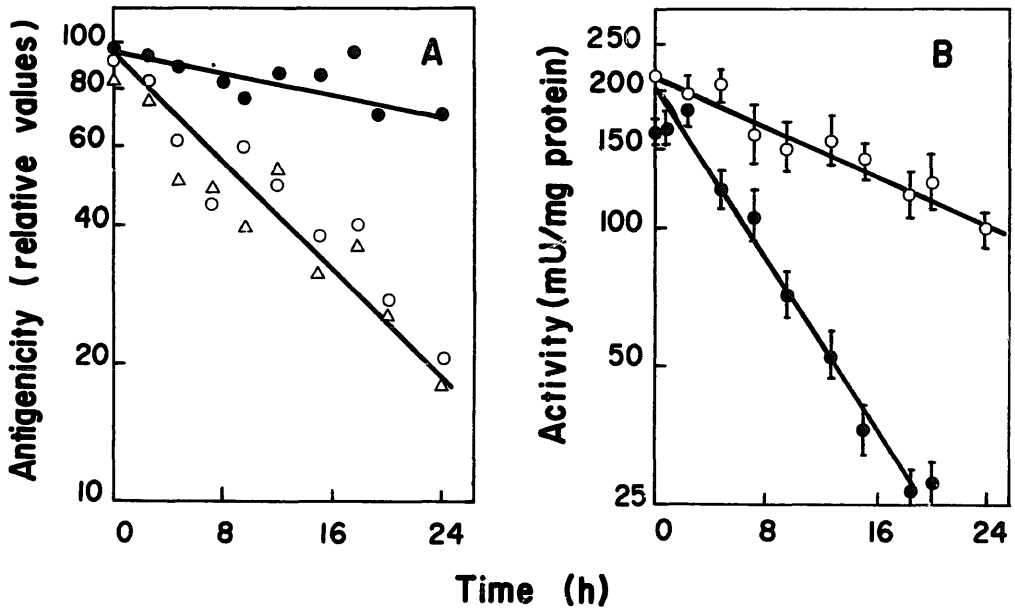

Fig. 1. Effect of the inhibition of protein synthesis on the content and activity of the plasma membrane ATPase. Cells were harvested during exponential growth on glucose and transferred to five times the initial volume of the media specified below. After incubation at 30°C for the indicated times, cells were harvested, washed and assayed for plasma membrane ATPase content using anti-ATPase antibodies and crude extracts (A) and plasma membrane ATPase activity at pH 6.5 and 2 mM ATP using crude plasma membrane preparations (non-fermenting cells)(B). The results are mean values of two or four experiments (those with standard desviation). Transfer media: ammonium-free medium containing 2% glucose (△); complete medium containing 10 μg/ml cycloheximide and 2% glucose (○) or 2% ethanol (●).

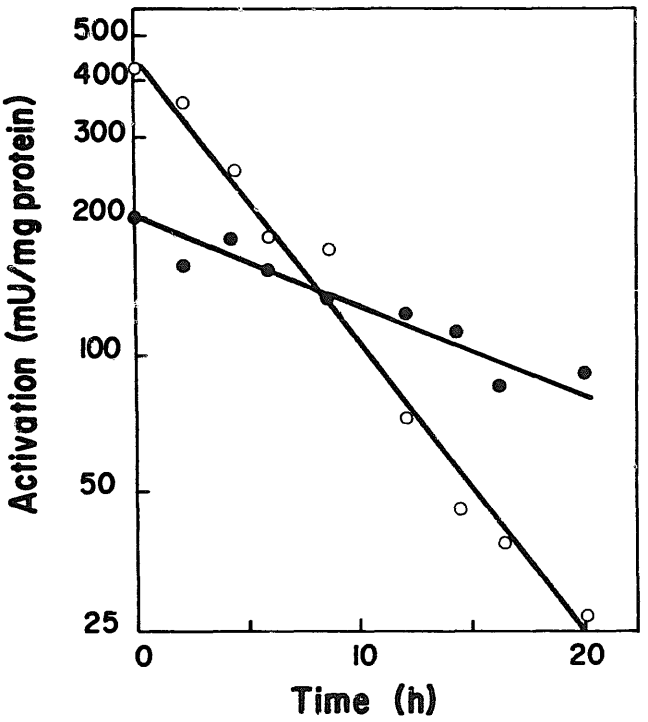

Fig. 2. Kinetics of the decrease of the activity of the ATPase activating system upon inhibition of protein synthesis. Cells were harvested and transferred to five times the initial volume of complete medium containing 10 μg/ml cycloheximide and 2% glucose (○) or ethanol (●). After incubation at 30°C for the indicated times, ATPase was assayed at pH 5.7 and 2 mM ATP in crude plasma membrane preparations of fermenting and non-fermenting cells and the activation factor calculated as in Table I.

presence of ethanol suggest that, in addition to these activation processes, an inactivation of the yeast plasma membrane ATPase activity is also possible. We have observed (Fig. 1A,B) that in the presence of ethanol, the ATPase activity decreased ($T_{\frac{1}{2}} = 8$ h) whereas the ATPase content remained constant ($T_{\frac{1}{2}} > 30$ h). A negative control of this kind has been previously reported during late exponential growth of yeast cells [22]. Probably it would take place when the need for H^+ efflux becomes lower than the actual capacity of the ATPase. In fact a low acid production is expected in the conditions used, that is, during utilization of ethanol by resting cells.

To investigate the turnover of the ATPase activating system we have studied the effect of the inhibition of protein synthesis on the three functions of this system: (i) Activation of ATPase in the presence of fermentation, (ii) increase in the optimum pH value of this enzyme, (iii) increase in its affinity for ATP. The results show that addition of cycloheximide or deprivation of a nitrogen source to glucose growing yeast produced a great decrease in these three functions (Table I). They also show that activation by fermentation decreased following first order kinetics indicating a half-life of about 5 h for the ATPase activating system (Fig. 2). When, instead of glucose, ethanol was present as energy source inhibition of protein synthesis produced a smaller effect and in this case a half-life of about 14 h could be calculated (Table I, Fig. 2).

In conclusion, the results shown in this work suggest that the yeast plasma membrane ATPase and its activating system show, respectively, a half-life of about 11 h and 5 h when protein synthesis is inhibited and a fermentable substrate is present in the medium. The fact that, in addition to ATPase, all other plasma membrane proteins so far examined [3–7] show low half-life

TABLE I

Effect of the inhibition of protein synthesis on the three functions of the ATPase activating system

Treatment [a]	Time of [b] treatment (h)	Substrate in the medium									
		glucose [a]					ethanol [a]				
		activation [c] (mU/mg protein)	K_m [d] (mM)		Optimum [e] pH		activation [c] (mU/mg protein)	K_m [d] (mM)		Optimum [e] pH	
			NF	F	NF	F		NF	F	NF	F
Ammonium starvation	0	480	6.7	0.7	5.5	6.5	200	4.0	0.7	5.6	6.5
	16	30	4.0	2.5	5.7	5.7	105	12.0	0.8	5.6	6.5
Addition of cycloheximide	0	450	6.7	0.7	5.7	6.3	190	6.7	1.0	5.7	6.5
	16	40	4.5	2.7	5.7	6.0	90	12.0	6.0	5.7	6.5

[a] Cells were harvested during exponential growth on glucose and transferred to three times the initial volume of the ammonium-free medium containing 2% glucose or ethanol, or complete medium containing 10 μg/ml cycloheximide and 2% glucose or ethanol as indicated.

[b] After incubation at 30°C for the indicated times, cells were harvested and washed (non-fermenting, NF) or treated with glucose (fermenting, F) as in Ref. 11.

[c] ATPase activity was assayed in purified plasma membrane preparations. Activation is the difference between the activity of fermenting and non-fermenting cells at pH 5.7 and 2 mM ATP.

[d] ATPase activity was assayed in purified plasma membrane preparations at pH 5.7 and ATP concentrations ranging from 0.2 to 8 mM. The apparent K_m were extrapolated from double-reciprocal plots.

[e] ATPase activity was assayed in purified plasma membrane preparations at 2 mM ATP and pH ranging from 5.0 to 8.0. Similar values were obtained with two different membrane preparations.

values as compared with the bulk of proteins in this organism [1,2], suggest that a rapid proteolysis in resting cells could be a characteristic of these proteins. On the other hand, the fact that the ATPase activating system behaves similarly to plasma membrane proteins could indicate that this system is placed in this membrane.

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