Conditions of activation of yeast plasma membrane ATPase

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The in vivo activation of the H^+-ATPase of baker's yeast plasma membrane found by Serrano in 1983 was demonstrated with D-glucose aerobically and anaerobically (as well as in a respiration-deficient mutant) and, after suitable induction, with maltose, trehalose, and galactose. The activated but not the control ATPase was sensitive to oligomycin. No activation was possible in a cell-free extract with added glucose. The ATPase was not activated in yeast protoplasts which may account for the absence of glucose-stimulated secondary active transports in these wall-less cells and provide support for a microscopic coupling between ATPase activity and these transports in yeast cells.

ATPase *Plasma membrane* *Oligomycin sensitivity* *Baker's yeast* *Protoplast* *Proton symport*

1. INTRODUCTION

The remarkable activation of plasma membrane H^+-ATPase of yeast by glucose in situ [1] was examined here with a view to its substrate specificity and its positive role in proton symports.

2. MATERIALS AND METHODS

2.1. *Yeast strain and its cultivation*

Saccharomyces cerevisiae K (CCY 21-4-60) was grown for 20 h at 30°C in a mineral medium [2] containing 0.8% D-glucose (or D-galactose or maltose) and 0.3% Difco yeast extract. *S. cerevisiae* DXIIA (CCY 21-4-19) with a deletion in the mitochondrial genome was grown for 24 h in the same medium. After harvesting, the yeast was washed twice with distilled water.

2.2. *Preparation of membranes*

Yeast cells (100–150 mg fresh wt/ml) were suspended in a medium containing 0.1 M 2-(morpholino)ethanesulfphonic acid (Mes), adjusted with Tris to pH 6.5, and incubated aerobically or anaerobically (under highly purified argon) with mild agitation at 30°C. After 5 min, different sugars were added to a concentration of 0.1 M and, after a further 5 min of incubation, the suspensions were frozen. The total membrane fraction was prepared as in [1].

2.3. *Preparation of protoplasts*

Cells (about 400 mg fresh wt/ml) were treated with dried snail-gut juice (prepared in this laboratory from garden-snail stomachs) in the presence of mercaptoethanol [3] and the resulting protoplasts were kept in 0.7 M mannitol. The crude membrane fraction was prepared as from whole cells.

2.4. *Preparation of cell-free extract*

One g fresh wt of yeast was suspended in 4 ml of 0.1 M Mes at pH 6.5, glass beads (0.5 mm in diameter) were added (8 ml) and Tris to 25 mM and EDTA to 5 mM. The crushed material (after removal of glass beads) was suspended in 6 ml of 20% glycerol, 10 mM Tricine (pH 7.5), 0.1 mM EDTA and 0.1 mM dithiothreitol. Centrifugation at 5000 × *g* for 3 min and again at 100000 × *g* for 30 min yielded a particle-free supernatant which was used for experiments.

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2.5. *ATPase estimation*

The ATPase activity was determined principally as described in [1]. Briefly, 50–80 μg of total membrane fraction was resuspended in 2 ml medium containined 50 mM Mes (adjusted with Tris to pH 5.7), 5 mM sodium azide, 0.2 mM ammonium molybdate, 10 mM $MgSO_4$, 50 mM KCl and 0.1 mg egg yolk lysolecithin (Sigma type I) per ml. Where indicated, different inhibitors were added. After 5 min incubation at 30°C the reaction was started with 20 μl of 0.1 M ATP (pH 6.5, adjusted with NaOH). One-ml samples were removed after 5 and 10 min and inorganic phosphate was determined as in [4]. One-ml samples were added to 1 ml ice-cold trichloroacetic acid (10%) and, after centrifugation for 3 min at 4000 × *g*, 1.5 ml trichloroacetic acid extract was pipetted into 4 ml reagent, consisting of 1% ammonium molybdate and 0.014% potassium antimonyl tartrate in 1.25 M H_2SO_4, 1% ascorbic acid and concentrated acetic acid (2:1:1). The mixture was stirred vigorously and absorbance was read at 578 nm after 20 min.

2.6. *Protein estimation*

The amount of protein in samples during preparation was determined as in [5], with bovine serum albumin as standard.

2.7. *Materials*

Yeast extract was from Difco, Mes and potassium antimonyl tartrate were from Fluka (Switzerland), dithiothreitol and egg yolk lysolecithin were from Sigma (FRG), sodium ATP, diethylstilbestrol and oligomycin were from Serva (FRG).

3. RESULTS AND DISCUSSION

In general agreement with the results of Serrano [1] and in an extension thereof, D-glucose, but also maltose, trehalose and D-galactose after growth on suitable inducing sugars, were able to activate the H^+-ATPase (table 1).

It made no significant difference whether the preincubation with glucose was done aerobically or anaerobically or whether a respiration-deficient mutant was used. All this, together with the absence of activation by ethanol, indicates that the glycolytic part of sugar degradation provides the factor and/or energy for converting the ATPase to its higher-affinity form.

Table 1

ATPase activity in the plasma membrane fractions of *S. cerevisiae* after aerobic preincubation with different substrates

Preincubation with	Range of relative activity
(No preincubation)	1.0[a]
D-Glucose	3.0–4.2
Maltose[b]	2.4–3.0
Trehalose	1.0–1.2
Trehalose[b]	2.5–3.0
α-Methyl-D-glucoside	0.8–1.1
D-Galactose[c]	2.0–3.0
Ethanol	0.6–0.9
D-Glucose[d]	1.8–2.0

[a]The control had an activity of 0.17–0.22 μmol phosphate/min per mg protein
[b]After growth on maltose
[c]After growth on D-galactose
[d]Using a respiration-deficient mutant

The effects of vanadate, diethylstrilbestrol and oligomycin after preincubation with different substrates are shown in table 2. Whilst the inhibition by the first two compounds was almost exactly as that observed in [1] the action of oligomycin is a new and unexpected observation. This inhibitor of the F_0F_1 type of H^+-ATPase [6] was found to affect significantly more the activated ATPase (41% on average) than the inactive one (13% on average). This is hardly the type of effect oligomycin exerts on the mitochondrial ATPase but might be related to its action on the dephosphorylation of the phosphoenzyme of the E_1E_2 types of ATPase [7].

To show whether ATPase can be activated by all the cell components in vitro, the ATPase-containing membrane preparation from non-activated cells was combined with a cell-free extract (capable of glycolysis) to which glucose was added. Table 3 shows that no activation took place, apparently because certain structural relationships within the cell must be preserved for it to occur.

It was observed earlier [3] that yeast protoplasts do not display the stimulation of proton symports

Table 2

Level of membrane-bound ATPase activity (μmol phosphate/min per mg protein) after different preincubations of cells and its sensitivity to inhibitors added to the assay mixture

Preincubated with	ATPase activity			
	No inhibitor	Vanadate (10/μM)	Diethylstil-bestrol (10 μM)	Oligomycin (25 μM)
D-Glucose, aerobically	0.661	0.270	0.274	0.418
		59.1[a]	58.5	36.6
D-Glucose, anaerobically	0.650	0.313	0.151[b]	0.377
		51.8	76.7	41.9
Trehalose	0.220	0.170	0.098	0.190
		22.7	54.5	13.6
α-Methyl-D-glucoside	0.250	0.190	0.140	0.210
		24.0	44.0	16.0
Maltose[c]	0.648	–	–	0.408
		–	–	37.0
D-Galactose[d]	0.656	–	–	0.346
		–	–	47.3
Ethanol	0.140	0.110	0.066	0.120
		21.5	52.9	14.1
(No preincubation)	0.220	0.165	0.090	0.193
		25.1	59.1	12.5

[a]Percent inhibition
[b]20 μM diethylstilbestrol was used which, even aerobically, inhibits by 76.5%
[c]After growth on maltose
[d]After growth on galactose

of sugars and amino acids produced by preincubation with glucose or similar substrates in whole cells and the view was advanced [8] that this may be related to the greatly diminished H^+ extrusion by the protoplasts after adding glucose to the suspension.

Table 3

Activity of membrane-bound ATPase (in μmol phosphate/min per mg protein) after stimulation with glucose in vitro

Additions	Activity	
	Control	With 25 μM oligomycin
None	0.17	0.17
D-Glucose	0.58	0.47
Cell-free extract[a]	0.13	0.14
Cell-free extract plus glucose	0.14	0.14

[a]Membranes (3.05 mg protein) were combined with 3 ml cell-free extract

Now it appears that ATPase-containing preparations of protoplast membranes differ from those of whole cells in that their ATPase cannot be activated by glucose (table 4). That we are dealing here with a non-activated form of ATPase is further documented by the relatively small inhibition by vanadate and oligomycin. The high absolute values of ATPase activity per weight of protein may be due to some peripheral proteins being lost during removal of the cell wall with the snail-gut juice.

The conclusion one can draw from this is that it is only the activated form of H^+-ATPase that can provide the local pH and possibly membrane potential differences required for driving the various secondary proton symports in yeast. It serves as further evidence for the view [9] that the ATPase must be functioning for these transports to occur. Without its function, even if the overall measured pH difference is sufficient for driving a

Table 4

ATPase activity (in μmol phosphate/min per mg protein) of the total membrane fraction from yeast protoplasts

Inhibitor added	I	II	III
None	0.698	0.712	0.602
Vanadate (10 μM)	0.501	0.548	0.526
	28.3[a]	23.0	12.5
Diethylstilbestrol (10 μM)	0.251	0.236	0.197
	64.0	66.8	67.2
Oligomycin (25 μM)	0.639	0.560	0.558
	8.5	21.3	7.2

[a]Percent inhibition

I, protoplasts preincubated in mannitol buffer alone; II, protoplasts preincubated in mannitol buffer with 1% D-glucose; III, cells incubated with 1% D-glucose before preparation of protoplasts (again in the presence of glucose)

given proton symport, it will not proceed [10]. The absence of these transports is not due to the respective proteins not being synthesized as protein synthesis, as well as induction of systems such as that for D-galactose, proceed normally in protoplasts.

In this respect the present situation may represent an analogy to the mosaic membrane hypothesis [11] advanced for the coupling between substrate oxidation and ATP synthesis in mitochondria. One may speculate that the H^+-ATPase is in direct, perhaps molecule-to-molecule, contact with the proton-driven transport systems.

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