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Partial Characterization of the Plasma Membrane ATPase from a *rho*^{0} Petite Strain of *Saccharomyces cerevisiae*

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Abstract

Crude membrane preparations of a *rho*^{0} mutant of *Saccharomyces cerevisiae* exhibit Mg^{2+}-dependent ATPase activity. Over the optimal pH range, 5.0–6.75, the apparent V_{max} of the enzyme equals 590 nmoles of ATP hydrolyzed per minute per milligram protein, with an apparent K_m for ATP of 1.3 mM. ATP hydrolysis is insensitive to ouabain, venturicidin, aurovertin, and the protein inhibitor described by Pullman and Monroy; inhibited by oligomycin (at high concentrations) and sodium orthovanadate, and it is sensitive to dicyclohexylcarbodiimide, *p*-hydroxymercuribenzoate, hydroxylamine, sodium fluoride, and sodium iodoacetate. The pH optimum and the inhibitor pattern distinguish the plasma membrane enzyme from the mitochondrial F_1 ATPase still present in these cells (this activity is sensitive to efrapeptin, aurovertin, and the protein inhibitor, but resistant to DCCD). In addition, the activity of the plasma membrane enzyme and its affinity for ATP are responsive to changes in the composition of the growth medium, with the highest activity observed in cells grown on methyl-α-D-glucoside, a sugar which results not only in partial release from catabolite repression but also requires the induction of an active transport system for growth.

Introduction

The plasma membrane of yeast contains a Mg^{2+}-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity [2–8]. The properties of the enzyme

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responsible for this activity differ significantly from those of the mitochondrial ATPase with respect to pH optimum, subunit composition, and sensitivity to various inhibitors [4, 7, 8]. Dufour and Goffeau [9] succeeded in solubilizing and purifying the plasma membrane ATPase from *Schizosaccharomyces pombe* using lysolecithin and found it to be a protein of molecular weight 100,000 ± 5000. Its activity was insensitive to oligomycin and venturicidin, two characteristic inhibitors of the mitochondrial enzyme, but was inhibited by dicyclohexylcarbodiimide (DCCD),[2] an inhibitor of the protonophoric function of the mitochondrial enzyme, as well as by Dio-9, another mitochondrial inhibitor. The DCCD inhibition of this enzyme is important in view of observations which suggest that the plasma membrane of yeasts contains an active proton pump furnishing the driving force for the transport of metabolites such as amino acids or phosphate ions across the membrane [10–12].

In contrast, the analogous enzyme from *Saccharomyces cerevisiae* has not yet been purified and is not amenable to the lysolecithin procedure of Dufour and Goffeau ([9]; Goffeau, personal communication). In view of the postulated similarity of active transport and its underlying mechanism among different yeast species, we have taken advantage of the unique properties and availability of *rho*^{0} mutants of *S. cerevisiae* to study the nature of the plasma membrane ATPase in this species. Such mutants lack mitochondrial DNA [13, 14], and hence F_0, the tightly membrane-bound portion of the mitochondrial ATPase resulting in a deficiency in the protonophoric, energy-transducing aspect of the enzyme [15–20]. Use of them can obviate confusion about the nature of the ATP hydrolyzing activity that is sensitive to characteristic inhibitors of this protonophoric function, such as DCCD, and, in addition, should simplify purification of the enzyme.

While some of the properties of the enzyme in wild type cells have been described by Fuhrmann et al. [4] and more recently by Willsky [8], only sketchy data are available on the enzyme in *petite* (not necessarily *rho*^{0}) mutants [2, 21]. Therefore, in the present study we have determined the essential parameters of the plasma membrane enzyme from a *rho*^{0} mutant—kinetic constants, ion requirements, and spectrum of inhibitor sensitivity. Significantly, we find that the enzyme is not only sensitive to DCCD, suggesting its possible role in energy-dependent proton transport across the plasma membrane, but is also responsive to changes in the carbon source in the growth medium and the resulting extent of catabolite repression.

[2]Abbreviations used: CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; DNP, dinitrophenol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol-bis(β-aminoethyl)*N,N'*-tetraacetic acid; IAA, iodoacetic acid; *p*-Hg-Bz, *para*-hydroxymercuribenzoate; Tris, tris(hydroxymethyl)aminomethane.

Experimental

Strain and Culture Conditions

Saccharomyces cerevisiae, strain D243-4A (*a adel lys2 rho*^{0}, originally obtained from Dr. Richard Criddle), was used throughout these studies. This petite mutant was induced by means of ethidium bromide, and its properties have been extensively documented by Hall et al. [14]. Cells were grown at 30°C in YPD medium (1% yeast extract, 1% Bacto-peptone, 2% glucose) and harvested by centrifugation (2000 × *g*, 10 min at 20°C) when they had reached an absorbance of 0.6–1.0 (600 nm, Bausch and Lomb Spectronic 20, cell density 1.7–3.0 × 10^7 cells/ml). The harvested cells were washed twice by resuspension in water and centrifugation (2000 × *g*, 10 min at 20°C) and finally resuspended to a concentration of approximately 0.5 g/ml (wet weight) in 0.25 M sucrose–0.05 M Tris, pH 7.5, and broken with glass beads in a Braun homogenizer (2 × 1 min). The broken cells were then pelleted at 4°C for 5 min at 1000 × *g* and the supernatant was removed and pelleted at 4°C for 40 min at 12,000 × *g*. The pellet was resuspended in 0.25 M sucrose–0.05 M Tris (pH 7.5) to a concentration of approximately 5 mg protein/ml and was used as a source of particulate enzyme throughout this study.

Assay of ATP Hydrolysis

ATP hydrolysis was measured spectrophotometrically at 30°C by observing the decrease in absorbance at 340 nm using a NADH-linked ATP regenerating system [22]. The reaction mixture contained the following in a final volume of 1.01 ml: 25 mM Tris-acetate (pH 5.5), 12 mM $MgCl_2$, 2.5 mM phosphoenolpyruvate, 1 mM KCN, 0.17 mM NADH, 100 μg of pyruvate kinase, 15 μg of lactate dehydrogenase, 6 mM ATP, and approximately 50 μg of pellet protein. The reaction mixture also contained 5% glycerol (due to the use of 50% glycerol for the pyruvate kinase and lactate dehydrogenase stock solutions). The assay medium was made up fresh daily, stored on ice in the dark, and used within 2 h. That ATP hydrolysis was the rate-limiting step in this assay was confirmed by control experiments in which hexokinase and glucose were added to the reaction mixture in the absence of particulate protein to produce a rate of absorbance change approximately equal to the highest rate measured with added protein. Under these conditions doubling the amount of hexokinase doubled the rate. All rate measurements were made during a period of linear absorbance change as recorded with a Zeiss M4QII spectrophotometer with a thermostatically controlled, water-jacketed cuvette holder connected to a Gilford recorder (Model 6040). Rates of absorbance change were linear for at least 5 min. In some

experiments with inhibitors it was necessary to add organic solvents at concentrations as high as 1% to the assay. In these cases appropriate controls were run and all results corrected accordingly.

Protein Determination

Protein was determined by the method of Lowry et al. [23] using bovine serum albumin as a standard. Determinations were made in the pellet resuspension medium.

Chemicals

ATP (vanadate-free), phosphoenolpyruvate, NADH, pyruvate kinase, lactate dehydrogenase, and oligomycin were all from Sigma Chemical Co. Sodium orthovanadate was from Matheson, Coleman and Bell; *p*-hydroxymercuribenzoate from Aldrich Chemical Co.; DCCD from Baker; ouabain from Calbiochem; hydroxylamine from Fisher Scientific; and venturicidin from BDH Biochemicals. Efrapeptin was a gift from Eli Lilly Co. The inhibitors 1799 and carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) were gifts from Dr. Heytler of E.I. duPont de Nemours Company. Aurovertin was a gift from Dr. H. Penefsky and the F_1 inhibitor of Pullman and Monroy [1] was kindly supplied by Dr. M. Pullman. All other reagents were of the highest purity commercially available.

Results

Kinetic Parameters of the [Mg^{2+}]-ATPase

Particulate preparations from the *rho*^{0} mutant of *S. cerevisiae* were capable of hydrolyzing ATP under our assay conditions. As determined from the Lineweaver–Burk plot shown in Fig. 1, this activity has an apparent V_{max} of 590 nmol ATP hydrolyzed per minute per milligram pellet protein and an apparent K_m for ATP of 1.3 mM.

Cation Requirements

The ATPase activity present in the particulate fraction is dependent upon the presence of Mg^{2+} ions (Fig. 2B). Increasing the Mg^{2+} concentration from 0 to 6 mM (equal to the ATP concentration in the reaction medium) resulted in increasing stimulation of activity, while at concentrations above 12 mM the extent of stimulation decreased. Mn^{2+} ions could substitute for Mg^{2+}, although at concentrations greater than 6 mM the degree of stimulation decreased markedly (Fig. 2B). On the other hand, calcium ions could not

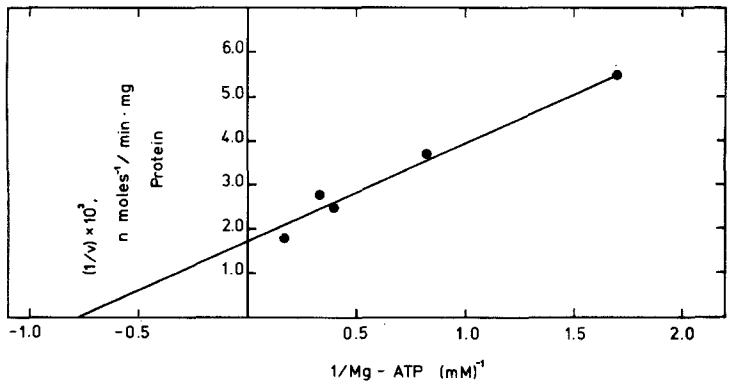

Fig. 1. Double-reciprocal (Lineweaver–Burk) plot showing the dependence of reaction rate on ATP concentration. Assays were performed at 30°C and pH 5.5. Each point represents the average of two to three determinations from two separate experiments. The gross standard error of the mean for all samples was approximately ±6%. The line indicated was fitted by linear regression analysis.

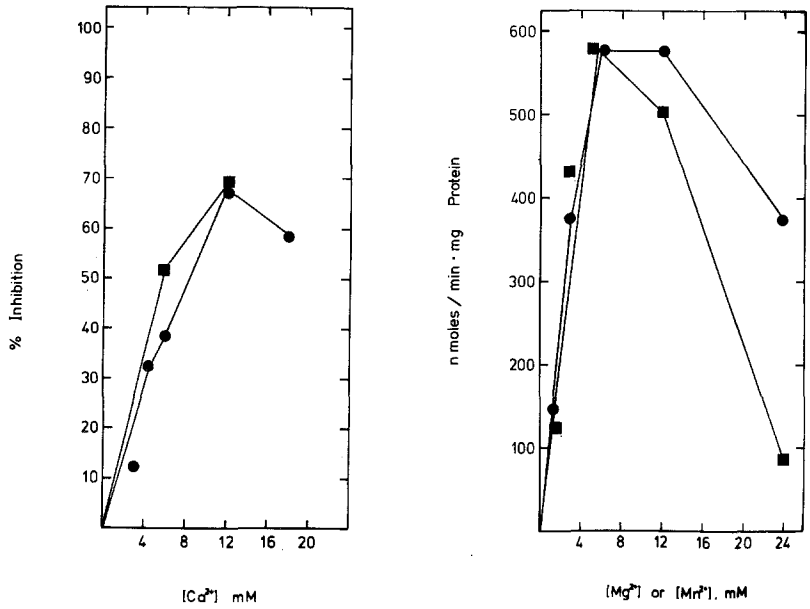

Fig. 2. Cation dependence of the ATPase activity. All assays were performed at 30°C and pH 5.5 as described under Experimental. $MgCl_2$, $MnCl_2$, and $CaCl_2$ were made up in water. (A) Inhibition of activity by Ca^{2+}. Assays were carried out in the presence of 12 mM Mg^{2+} (●) or 6 mM Mn^{2+} (■). (B) Mg^{2+}, Mn^{2+} dependence. ●, Mg^{2+}; ■, Mn^{2+}.

replace magnesium, with no stimulation by Ca^{2+} alone at concentrations as high as 18 mM. However, when Ca^{2+} was added to the incubation mixture in the presence of either 12 mM Mg^{2+} or 6 mM Mn^{2+}, a Ca^{2+} concentration-dependent inhibition of activity was observed (Fig. 2A). The addition of potassium or sodium ions (3–12 mM), singly or in combination, had no effect on activity, with or without Mg^{2+} in the reaction mixture.

Effect of Anions

A number of anions were tested for possible effects on Mg^{2+}-dependent ATPase activity. As shown in Table I, at the standard assay pH of 5.5, bisulfite ions inhibited enzyme activity strongly; succinate, tartrate, and formate ions had only a slight effect while bicarbonate ion stimulated activity relative to acetate. When the same experiment was conducted at or above pH 8.0 (see below and Fig. 3), somewhat different results were obtained. In this case, only bisulfite was an effective inhibitor; tartrate and bicarbonate both stimulated activity somewhat, while succinate and formate ions had no significant effect (Table I).

pH Dependence

When assayed in the standard Tris-acetate buffer, maximal ATPase activity was obtained between pH 5.0 and pH 6.75, with lower but significant levels also found above pH 8.0 (Fig. 3). In order to assess the relationship between the activities present in these two regions, we determined their sensitivity to efrapeptin, aurovertin, DCCD, and to the protein inhibitor of Pullman and Monroy [1] (see below and Table II). The ATPase pH region around 5.5 was strongly inhibited by DCCD and relatively insensitive to the other inhibitors, while the converse was true at pH 8 and above (Fig. 3). In particular, at intermediate pH values the sensitivities to efrapeptin and

Table I. Effect of Anions on Plasma Membrane ATPase Activity[a]

Anion	Relative ATPase activity	
	pH 5.5	pH 8.0
Acetate	100	78
Succinate	87	79
Tartrate	88	96
Bisulfite	15	26
Bicarbonate	132	91
Formate	88	75

[a]Assays were performed in 25 mM Tris buffer containing the anions indicated (25 mM final concentration). All activities are presented relative to the activity at pH 5.5 in Tris-acetate, which was arbitrarily given a value of 100. Results are means from duplicate samples of two separate experiments.

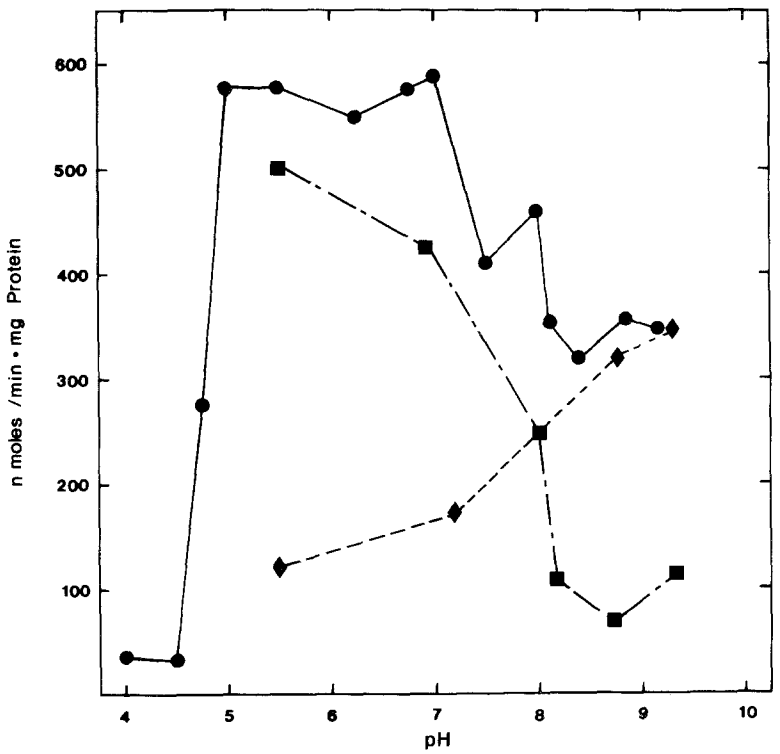

Fig. 3. pH dependence. Assays were performed at 30°C in Tris-acetate buffer. Inhibitors were tested on fresh samples by incubating enzyme, buffer, and either DCCD (200 μM, ♦) or efrapeptin (75 nM, ■) for 10 min at 30°C, then adding the remaining constituents of the assay mixture and proceeding as described in Experimental. Where required, values are corrected for effects of the solvent (methanol) used to dissolve the inhibitors. Each point represents the mean of duplicate samples from three to nine separate experiments. The standard error of the mean for the pH curve was ±8% and for the inhibitor curves was approximately ±10%.

Table II. Effect of Aurovertin and the Protein Inhibitor of Pullman and Monroy on ATPase Activity[a]

Inhibitor	Concentration	% Inhibition	
		pH 5.5	pH 8.0
Aurovertin	0.41 mM	0	50
Protein Inhibitor	43 μg/ml	0	51
	86 μg/ml	0	58

[a]Membranes (50 μg) were preincubated for 10 min in 5 mM imidazole buffer (pH 7.0) containing 0.5 mM ATP, 0.5 mM $MgCl_2$, 1% methanol, and inhibitor in a final volume of 230 μl. The ATPase reaction mixture was then added and ATP hydrolysis monitored at the two pH values at 30°C as described in Experimental. Imidazole buffer was used during the preincubation period because Tris has been shown to diminish the effect of the protein inhibitor [1].

DCCD showed inverse, coordinate changes; sensitivity to DCCD decreased while that to efrapeptin increased as the assay pH became more alkaline.

Effect of Inhibitors

A number of potential inhibitors of ATPase activity were tested for their effect on enzymatic activity. These assays were all carried out at pH 5.5 in Tris-acetate buffer. The activity of the membrane preparation was insensitive to ouabain (0–5 mM) as well as to efrapeptin (0.02–1.0 μg/ml), aurovertin (50–410 μM, Table II), the protein inhibitor of Pullman and Monroy [2] (Table II), and venturicidin (5–100 μg/ml). The last four are all potent inhibitors of the mitochondrial ATPase with their targets its F_1 (aurovertin, efrapeptin, protein inhibitor) and F_0 (venturicidin) portions [24, 25]. On the other hand, oligomycin, another very effective inhibitor of F_0, inhibited the particulate ATPase by 30–40% at concentrations above 10 μM. This concentration is significantly above that required for inhibition of the mitochondrial enzyme in wild-type cells [26, 27], but similar to that reported for the "pH 6.2" enzyme of crude mitochondrial membranes by Kovac and Weissova [28]. These observations, together with the fact that protonophoric mitochondrial ATPase is absent from petite strains, suggest that the inhibition by oligomycin (Fig. 4) at high concentrations may be due to a nonspecific effect of the inhibitor either directly on nonmitochondrial ATPase [29], or indirectly through the membrane environment of the enzyme.

Inhibition was also observed with uncouplers of oxidative phosphorylation. Dinitrophenol (DNP), carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) and 1799 all inhibited ATPase activity by about 30% at a concentration of 50 μM. At much higher concentrations (500 μM) DNP inhibited activity completely. In contrast, DNP (50 μM) inhibited the pH 8.0 activity by 51%.

A number of other compounds also inhibited particulate ATPase activity. *para*-Hydroxymercuribenzoate was an effective inhibitor, producing 50% inhibition at a concentration of approximately 6 μM and 90% inhibition at concentrations above 10 μM (Fig. 4). Sodium orthovanadate, a potent inhibitor of other ATPases and phosphotransferases [30, 31], produced half-maximal inhibition at a concentration of approximately 10 μM (Fig. 5). This inhibition was overcome in a concentration-dependent fashion by EDTA, which was complete at 1 mM. Similar results have been reported by Bowman and Slayman [32] for the plasma membrane ATPase from *Neurospora crassa*. EGTA, on the other hand, was ineffective in reversing vanadate inhibition (data not shown). The hydrophobic carboxyl reagent DCCD was found to be an effective inhibitor (Fig. 5). This inhibition appeared confined to the plasma membrane component, with no significant effect on the mitochondrial (F_1) enzyme. At concentrations above 200 μM it produced

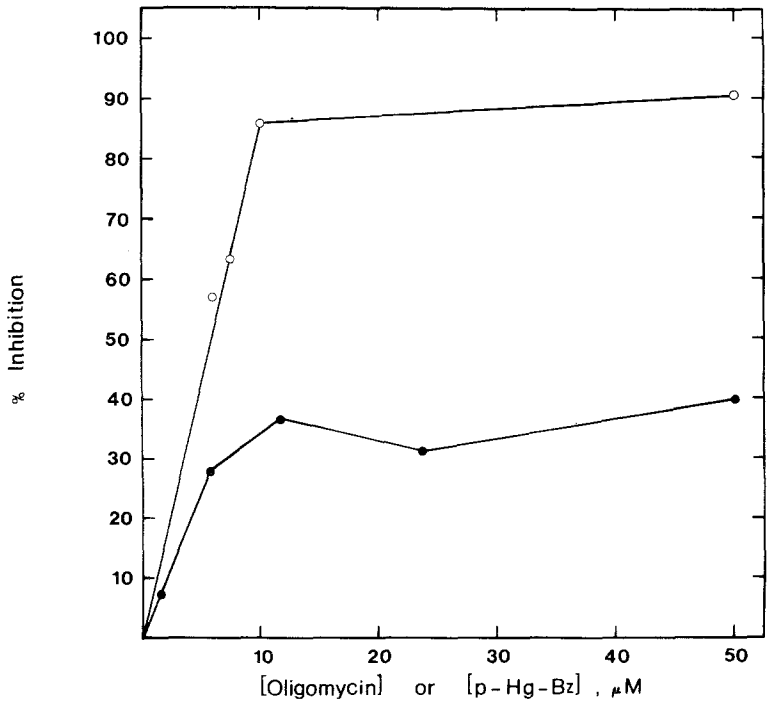

Fig. 4. Inhibition of the membrane-bound ATPase by oligomycin (●) and *p*-hydroxymercuribenzoate (○).

greater than 80% inhibition, while at 25 μM it still inhibited by 50%. Enzyme activity was also inhibited completely by hydroxylamine (40 mM), a compound that has been shown to cause dephosphorylation of the (acyl) phosphoprotein associated with the plasma membrane ATPase [8]. Sodium fluoride and sodium iodoacetate (IAA), used frequently as inhibitors of glycolysis, were less effective (50% inhibition at 3.4 mM and 0.6 mM, respectively) but did show greater than 90% inhibition at concentrations above 9 mM (NaF) and 4 mM (IAA).

Effect of Alternate Carbon Sources

In order to determine whether ATPase activity was responsive to changes in the composition of the growth medium, cells were grown in YP medium containing, at a final concentration of 2%, either galactose or methyl-α-D-glucoside. Galactose was chosen because it is a relatively weakly repressing carbon source (reviewed in [13]), and methyl-α-D-glucoside because it is both less repressing than glucose as well as actively transported [33]. Particulate preparations were isolated by a modification of the proce-

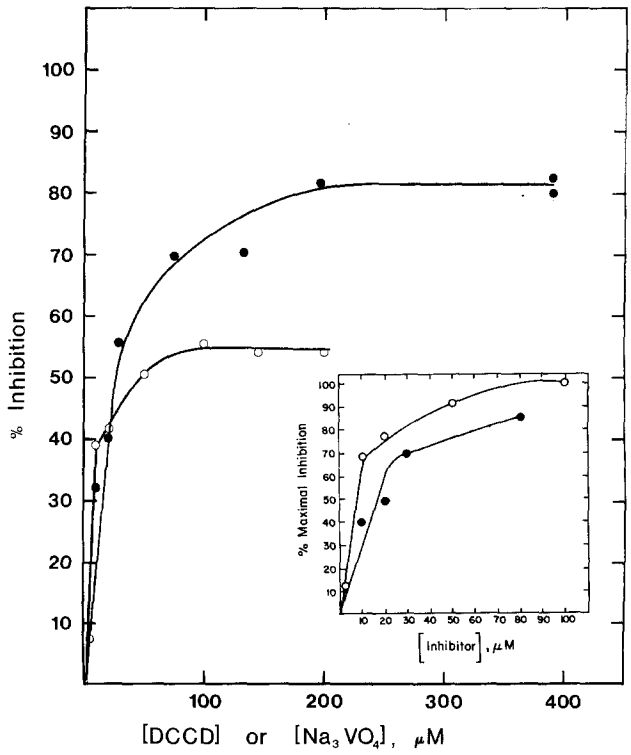

Fig. 5. Inhibition of the membrane-bound ATPase by DCCD (●) and sodium orthovanadate (●). Incubation and assay procedures were as described in the legend to Fig. 3. In the case of DCCD, values were corrected for the solvent (methanol) used to dissolve the inhibitor. Each point represents the average of at least two determinations from separate experiments. Insert: Re-plot of the region of the curve from 0–100 μM inhibitor.

dure described for cells grown on glucose. Cells grown on galactose or methyl-α-D-glucoside were more difficult to break than were cells grown on glucose. Therefore, for these experiments the breaking time was extended to 4 min in all cases. While this treatment provided thorough breakage, it also resulted in a lower apparent V_{max} for glucose-grown cells, presumably due to the presence of increased amounts of extraneous protein. The apparent K_m for the enzyme in these cells was unchanged. ATPase activity of these preparations measured at both pH 5.5 and pH 8.0 showed significant differences (Table III). Cells grown on methyl-α-D-glucoside exhibited approximately 3-fold greater activity (pH 5.5) than cells grown on glucose, as reflected in the apparent V_{max}, while, relative to glucose, galactose-grown cells showed a smaller increase. There were also notable differences in the apparent K_m

Table III. Effect of Alternate Carbon Sources on ATPase Activity[a]

Sugar Addition to the Medium	ATPase activity		
	K_m (mM)	V_{max} (nmoles/min/mg)	pH 5.5/pH 8.0
Glucose[b]	1.3	590	1.2
Glucose	1.2	123	1.1
Galactose	0.3	186	0.4
Methyl-α-Glucoside	0.5	324	0.5

[a]Cells were grown to a density of 1.7–3.0 × 10^7 cells/ml at 30°C in YP medium containing the sugars listed at a final concentration of 2%. They were then harvested, broken, and assayed as described in the text. Results are means from at least three separate experiments.
[b]Glucose cells broken as described in Methods. See Fig. 1.

values among these three preparations, with glucose-grown cells having the highest apparent K_m and galactose-grown cells the lowest. In addition, the ratio of activities at pH 5.5/pH 8.0 (assayed at the standard ATP concentration of 6 mM) fell from 1.1 for glucose-grown cells to 0.4 and 0.5 for galactose and methyl-α-D-glucoside-grown cells, respectively.

Discussion

In previous publications we (Jaynes and Mahler [34], and in preparation) and others [3, 4, 6, 8, 35, 67] have reported on the purification of plasma membranes from *S. cerevisiae* and the presence there of a Mg^{2+}-dependent ATPase. In the present studies, our strategy has been to utilize a *rho*^{0} mutant as a source of membranes; such cells are deficient in a mitochondrial energy-transducing system by virtue of the absence of the proton pumping device of its ATPase. However, a Mg^{2+}-dependent activity capable of hydrolyzing ATP is present in crude plasma membranes isolated from this strain, at levels comparable to those encountered in a similar fraction from its wild type (*rho*$^+$) parent (data not shown). The apparent V_{max} for the enzyme at pH 5.5 is more than an order of magnitude greater than the activity reported for membranes of wild type *S. cerevisiae* by Fuhrmann et al. [4], and compares favorably with that reported by Willsky [8] for the enzyme in membranes of wild type cells, grown on glucose. The activity of the *rho*^{0} mutant is within an order of magnitude of that described for the crude membrane fraction from *S. pombe* [7]. More significant is the good agreement observed for the apparent K_m of the membrane-bound enzymes in the various strains of yeast, as well as in *Neurospora crassa* [36, 37], all of which lie within the range of 1.3 to 1.7 mM.

Even though a crude membrane preparation was used as a source of enzyme throughout this study, several lines of evidence rule out the possibility

that the activity measured at pH 5–6 is due to extensive contamination by the mitochondrial ATPase. First, and significantly, we have chosen to study a *rho*^{0} mutant which lacks mitochondrial DNA and therefore a functional F_0 component of the mitochondrial enzyme. This mutant should still possess the catalytic F_1 component and, indeed, we observe Mg^{2+}-dependent ATP hydrolysis at pH 8–9 that is sensitive to F_1 inhibitors, such as aurovertin, efrapeptin, or the protein inhibitor of Pullman and Monroy [1], and resistant to DCCD, which binds to the F_0 segment of the mitochondrial enzyme [19, 38]. This behavior is in agreement with that observed by Kiehl and Hatefi [39] for beef heart mitochondrial complex V but in contrast to the reports of Pougeois et al. [40] for highly purified F_1 from beef heart and of Shoshan and Selman [41] for chloroplast-coupling factor I. Pougeois et al. [40] also show a pH dependence for inactivation of F_1 by high concentrations of DCCD; preincubation of F_1 with DCCD at pH 6 to pH 9 resulted in decreasing inactivation with virtually no inactivation above pH 8.5. When the preparation described here was preincubated at pH 7.0 with as much as 3 mM DCCD, and then assayed at pH 8 or above, only minimal (<30%) inhibition was observed (data not shown). Inhibition by this agent under our standard conditions therefore probably reflects an intrinsic property of the plasma membrane rather than contamination by the mitochondrial enzyme. This "high pH" activity thus appears to be due to residual mitochondrial F_1 ATPase. In contrast, the pH 5–6 activity is sensitive to DCCD and resistant to efrapeptin, aurovertin, and the protein inhibitor. The data of Fig. 3 and Table II permit an estimate of the extent of the mitochondrial (F_1) contamination at pH 5–6: the true plasma membrane activity at pH 5.5 is estimated at ≥85% of the total measured based on efrapeptin inhibition and virtually 100% based on inhibition by either aurovertin or the protein inhibitor. Willsky [8] was also able to distinguish between mitochondrial and plasma membrane ATPases by their differential sensitivities to efrapeptin, but could not utilize DCCD, since both enzymes in the *rho*$^+$ cells used in that study are inhibited by this compound. Although pH 5–6 activity is sensitive to high concentrations of oligomycin, it is resistant to venturicidin, another potent inhibitor of the mitochondrial holoenzyme. Therefore, on the basis of pH optima and sensitivity to various inhibitors we conclude that the enzyme activity at pH 5–6 present in the crude *rho*^{0} membrane pellet residues predominantly, if not solely, in the plasma membrane. It thus constitutes a separate entity, distinct from the mitochondrial enzyme, as already suggested by the preliminary study of Schatz [21].

Several other features of the pH 5–6 enzyme are also worth noting. First, although it is neither stimulated by sodium or potassium—singly or in combination—nor inhibited by ouabain, the enzyme is nevertheless inhibited to a significant extent by low concentrations of orthovanadate (i.e., vanadium

in the +5 oxidation state), an inhibitor of the plasma membrane (Na^+-K^+)- and Ca^{2+}-dependent ATPases of the red cell [30, 31, 42] and of the Mg^{2+}-dependent enzymes in *N. crassa* [32] and wild-type *S. cerevisiae* [8]. Furthermore, Bowman and Slayman [32] have shown that vanadate inhibition of the *N. crassa* enzyme can be reversed by EDTA or norepinephrine, but not by EGTA. The effect is not due simply to removal of free Mg^{2+} ions but rather appears to result from complex formation between EDTA or norepinephrine and either vanadate or ATP. Similar results were obtained in the present study: in the presence of EDTA, but not EGTA, vanadate inhibition was completely prevented at Mg^{2+} concentrations of 6–12 mM. These results are consistent with a hypothesis that plasma membrane ATPases—whether Na^+-K^+-, Ca^{2+}-, or Mg^{2+}-dependent—share common structural and catalytic features, perhaps involving phosphorylated intermediates [8, 43].

Second, although the *S. cerevisiae* enzyme, whether derived from wild-type [8] or *rho*^{0} strains, is similar to that of *S. pombe* with respect to cation dependence, pH optimum, and general spectrum of inhibitor sensitivity, it appears distinct by a number of criteria. Concentrations of Mg^{2+} above 12 mM inhibit the *S. cerevisiae* enzyme but have no effect on that from *S. pombe*. A similar inhibition by supraoptimal concentrations of Mg^{2+} has been observed for the ATPase of *N. crassa* [36] and bacterial membranes [44, 45]. Furthermore, the *S. cerevisiae* enzyme is stimulated by bicarbonate ion and strongly inhibited by bisulfite ion while the *S. pombe* enzyme is unaffected by these ions [7]. Additionally, while enzymes from both yeast species are inhibited by *p*-hydroxymercuribenzoate to approximately the same extent, the *S. cerevisiae* enzyme is more sensitive to oligomycin. The difference in solubility properties in lysolecithin as described in the Introduction also serves to distinguish the two enzymes.

The *N. crassa* and *S. cerevisiae* enzymes appear to be more sensitive to DCCD than that from *S. pombe*. DCCD is known to interact covalently with the hydrophobic subunit 9 of the mitochondrial ATPase complex and by so doing the block electrogenic proton flow [17, 46–49]. It has also been reported to react with purified beef heart F_1 [40] and with the Ca^{2+}-dependent plasma membrane ATPase from sarcoplasmic reticulum [50]. DCCD binding subunits have been purified from mitochondria [38, 40, 51–53], chloroplasts [54–56], and bacteria [57, 58] and found to have similar amino acid compositions and primary sequences [38]. In at least one case, the purified protein has been reconstituted into artificial lipid vesicles and demonstrated to act as a protonophore [54]. Thus, it is not unlikely that the plasma membrane ATPase of yeast which shares DCCD sensitivity with other transport ATPases is responsible for generating an electrochemical gradient across the membrane and is responsible for concomitant active

transport [4, 11, 59, 60]. An analogous function has also been ascribed to the enzyme in *N. crassa* [36, 61, 62].

Finally, the experiments on the changes in enzyme activity in response to alternate carbon sources (Table II) are of great interest. Such a change in energy source may be expected to affect cells in one of three, not mutually exclusive, ways: a) release from catabolite repression; b) induction of a transport system for the sugar in question, and c) coupling of (b) to membrane energization or another mechanism designed to convert it from facilitated diffusion to active transport. Any one of these three responses can affect or be affected by the plasma membrane and its ATPase.

Of the sugars studied, galactose is known to produce a relatively low level of catabolite repression [13] and to be taken up by an inducible, but energy-independent, facilitated diffusion mechanism [63, 64]. The levels of ATPase observed in cells grown on this sugar may therefore be taken as an expression of the response to item (a) and (b) above. The additional increment in enzyme activity when cells are switched from glucose to α-methyl-D-glucoside, a sugar known to be subject to active transport by an inducible system [33], probably driven by the electrochemical gradient across the plasma membrane [65], can therefore be tentatively equated to the response to item (c). Since, as discussed earlier, this gradient is now believed to be generated by the membrane ATPase [36, 60–62, 66], it is tempting to speculate that yeast cells contain a sensing element which links an increase in the supply of the enzyme to the plasma membrane to the presence in the medium of a carbon source requiring active transport across this barrier.

Note Added in Proof

R. Serrano has recently (*Eur. J. Biochem.*, **105** (1980) 419) reported that DCCD inhibits both Mg^{2+}-dependent ATPase activity and proton transport in a respiration-deficient strain of *S. cerevisiae*. F. Malpartida and R. Serrano have purified the plasma membrane Mg^{2+}-dependent ATPase from a wild-type strain of *S. cerevisiae* (*FEBS Lett.*, **111** (1980) 69).

Acknowledgment

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