Characterization of the Plasma Membrane Mg\textsuperscript{2+}-ATPase from the Yeast, *Saccharomyces cerevisiae*  

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The plasma membrane of *Saccharomyces cerevisiae* has a Mg\textsuperscript{2+}-dependent ATPase which is distinct from the mitochondrial Mg\textsuperscript{2+}-ATPase and at the pH optimum of 5.5 has a $K_m$ for ATP of 1.7 mm and a $V_{max}$ of 0.42 pmol of ATP hydrolyzed/mg/min. At least three protein components of the crude membrane ($M_r$ = 210,000, 160,000 and 115,000) are labeled with [$\gamma$-$^32P$]ATP at pH 5.5. These phosphoproteins form rapidly in the presence of Mg\textsuperscript{2+}, rapidly turn over the bound phosphate when unlabeled ATP is added, and dephosphorylate after incubation in the presence of hydroxylamine. Vanadate, an inhibitor of the Mg\textsuperscript{2+}-ATPase activity, blocks the phosphorylation of the 210,000- and 115,000-dalton proteins. At pH 7.0, only the 210,000- and 160,000-dalton proteins are phosphorylated. While these three phosphorylated intermediates have not been unambiguously identified as components of the Mg\textsuperscript{2+}-ATPase, the finding of such phosphorylated components in association with that activity implies that this enzyme differs in mechanism from the mitochondrial proton pump and that it is similar in mechanism to the metal ion pumps (Na\textsuperscript{+}-K\textsuperscript{-})-ATPase and Ca\textsuperscript{2+}-ATPase of the mammalian plasma membrane.

Two classes of membrane-bound ATPases have been described in the literature. Class I ATPases depend on Mg\textsuperscript{2+} for activity, are involved in oxidative phosphorylation, pump protons, and are composed of integral and peripheral proteins. These enzymes have at least six subunits and can hydrolyze ATP using two subunits of $M_r$ = 56,000 and 59,000. There is no phosphorylated intermediate involved in ATP hydrolysis. Examples of class I enzymes are the Mg\textsuperscript{2+}-ATPases of bacteria (1), chloroplasts (2), and mitochondria (3).

Class II ATPases are dependent upon Mg\textsuperscript{2+} for activity, and are also stimulated by other cations. They are involved in ion translocation and the maintenance of ion gradients, pump K\textsuperscript{+}, Na\textsuperscript{+}, or Ca\textsuperscript{2+}, and are composed of only integral membrane proteins. These enzymes have only one or two subunits and no phosphorylated intermediate involved in ATP hydrolysis. Examples of class II enzymes characterize the activity of the Mg\textsuperscript{2+}-dependent ATPase from the plasma membrane of *S. cerevisiae* with respect to its sensitivity to inhibitors, response to exogenously added cations, pH dependence, and ability to be stably phosphorylated by [$\gamma$-$^32P$]ATP. These experiments indicate that the enzyme is distinct from the mitochondrial Mg\textsuperscript{2+}-ATPase and the known mammalian metal ion pumps. In addition, the ATP hydrolytic activity of the enzyme has been associated with the formation of vanadate-sensitive phosphorylated intermediates, which implies that the *S. cerevisiae* plasma membrane Mg\textsuperscript{2+}-ATPase is similar in mechanism to the class II type plasma membrane ATPases.

**EXPERIMENTAL PROCEDURES**

**Materials**—*S. cerevisiae* strain S288c was a gift of D. Botstein at MIT. Glusulase was purchased from Endo Laboratories. The gel standards bovine serum albumin, phosphorylase b, and myoglobin were purchased from Sigma, while myosin was a gift of J. Anderson, Harvard University. Strophantidin, cycloheximide, oligomycin, phenylmethylsulfonyl fluoride, Tris/ATP for ATPase assays, and disodium ATP (grade II) for synthesis of [$\gamma$-$^32P$]ATP were products of Sigma. Sodium vanadate (Na\textsubscript{3}VO\textsubscript{4}) was obtained from Fischer Scientific Co. Carrier-free $^{35}$P in 0.2 M HCl was from New England Nuclear. Dio 9 was the gift of Koninklijke Nederlandsche Gist and Spiritus Fabriek (Delft, Netherlands), while efrapeptin was the gift of Robert Hamill (Eli Lilly).

**Cell Growth**—The yeast strains were maintained on slants of yeast extract/peptone/dextrose solid medium (13). The cells were grown at room temperature with gentle agitation in 20-liter batches of the medium described by Duell et al. (14) with addition of Antifoam A (Dow-Corning), and harvested with a Sharples centrifuge at about 15 h when they reached an absorbance at 550 nm of 1.7 to 2.2.

**Spheroplast Formation**—Spheroplasts were made using glusulase by a modification of the procedures of Wiley (15) and of Schatz and Kovalč (16). The cells were washed once in 0.3 M Tris, 40 mM EDTA (pH 9) and resuspended in three times their wet weight of 0.5 M sucrose, 0.2 M Tris and 20 mM EDTA (pH 9.0) before the addition of β-mercaptoethanol to a final concentration of 0.1 M. The cells were then incubated at 30°C for 20 min, sedimented by centrifugation at...
600 x g for 10 min, and washed once with Buffer A (0.5 M sucrose, 0.1 M sodium citrate, and 10 mM EDTA (pH 5.8)) before the addition of 0.2 ml glusulase/g wet weight of cells. After incubation for 30 to 90 min at 30°C when spheroplast formation was 70 to 95% complete (as followed by phase contrast microscopy), the glusulase reaction was stopped by chilling the cell suspension on ice. Spheroplasts were sedimented at 8000 x g for 10 min, the supernatant fluid was removed from the spheroplasts as described by Schatz and Kovanč (16), and the spheroplasts were then washed twice in Buffer A. Sodium tetrathionate, 1 mg/ml, and 2 mM PMSF (diluted from a freshly prepared 0.1 M stock solution in 100% ethanol) were added to the spheroplast suspension to inhibit proteolysis, while cycloheximide (25 ug/ml) was added to inhibit bacteriolysis if the spheroplasts were to be left overnight.

Membrane Isolation—Spheroplasts were sedimented by centrifugation at 10,000 x g for 10 min and resuspended in Buffer B (0.25 M sucrose, 5 mM EDTA, and 50 mM Tris-HCl, pH 7.2) before the addition of sodium tetrathionate and PMSF as described above. The spheroplasts were lysed with a Waring Blender at 0°C (using a total homogenization of 2 min in 200-bursts); cell debris, nuclei, and unbroken cells were removed by centrifugation at 1500 x g for 10 min and then the mitochondria were removed from the supernatant fluid by centrifuging twice at 15,000 x g for 10 min. The microsomal membranes were obtained from the resulting supernatant fluid by centrifugation at 85,000 x g for 2 hr and were gently resuspended to about 15 mg/ml protein (as measured by the Bailey modification of the Lowry protein assay (17)) in Buffer B using a glass homogenizer.

Sucrose Gradient Centrifugation—The buoyant density of the membrane fraction was determined by sucrose density centrifugation at 130,000 x g for 2 hr in a Beckman SW50 rotor. One millilitre of suspended membranes was layered over a 4.4-ml linear sucrose gradient (12 to 60% w/w containing 1 mM EDTA) resting on a cushion of 0.5 ml of 60% sucrose. Fractions were collected from the top of the gradient.

ATPase Assay—The ATPase assay of Goldin (18) using [γ-32P]ATP (prepared by the method of Glynn and Chappell (19)) was used with minor modifications in 50-μl reaction mixtures. Radioactivity was determined in Aquasol (New England Nuclear Corp.) by liquid scintillation counting. Since the hydrolytic activity at 37°C was linear with time up to 8 min and with protein concentration up to 0.6 mg/ml, assays were done at 37°C for 2 min with protein concentrations of 0.3 mg/ml.

Phosphorylation Procedure—The phosphorylation assay was a modification of procedures previously published (20–22). The 250-μl reaction mixture contained 10 mM Tris-HCl (pH 7.0) or 50 mM Tris/sucinate (pH 5.8), approximately 20 μM Tris/[γ-32P]ATP (specific activity 2 to 17 Ci/mmol) and 1.5 mg/ml of protein. Inhibitors and 5 mM MgCl2 were added as described in the figure legends and the reaction was always started by the addition of enzyme, terminated by the addition of 0.5 ml of cold 5% trichloroacetic acid, and subse- quently washed twice with 5 ml of cold wash fluid.

Hydroxyamine Treatment of the Phosphorylated Proteins—According to the procedure of Blatstein (50), the washed trichloroacetic acid precipitate was incubated in 0.08 M sodium acetate buffer, pH 5.8, in the presence or absence of 0.25 M hydroxyamine for 15 min at 0°C, collected by centrifugation and analysed by SDS gel electrophoresis.

Gel Electrophoresis—The SDS-polyacrylamide gel electrophoresis system using pH 2.4 phosphate buffer described by Barro and Avruch (23) was used throughout this study, and samples were prepared for electrophoresis by the method of Avruch and Fairbanks (24). Aliquots were taken for protein determination before the addition of β-mercaptoethanol to 0.2 M and the tracking dye, pyronin Y, and the samples were loaded onto the gel after 15 min at room temperature. Stains (50) (0.75 mm thick) were used for electrophoresis, each gel was dried using a Bio-Rad Model 224 slab gel drier, and autoradiography was done using Kodak SB-5 x-ray film. To quantitate the percentage of radioactivity present in each peak, the densitometer tracing was cut out of a autoradiograph of the original scan and weighed. The amount of radioactive phosphate in each peak was then calculated using the percentage of counts in each peak, the number of counts originally placed on the gel, and the specific activity of the [γ-32P]ATP.

Molecular Weight Determination—Molecular weights were determined using the gel electrophoresis system described above with the following gel standards: myoglobin (M, = 17,200), bovine serum albumin (M, = 65,000), phosphorylase b (M, = 94,000) and myosin (M, = 220,000). As the graph of the log of the molecular weight versus the relative mobility of the protein standards gave a straight line, the molecular weight of the phosphorylated intermediates were determined from this standard curve using the relative mobilities obtained from the autoradiogram for these proteins.

RESULTS

Characterization—The ATPase activity of a microsomal membrane fraction of the yeast S. cerevisiae is Mg2+-dependent. The optimum Mg2+ concentration of 5 mM corresponds to a Mg2+-ATP ratio of approximately 1 and a large excess of Mg2+ (25 mM) causes only a 10 to 20% inhibition of the enzyme (Fig. 1). The dependence of ATPase activity on the concentration of ATP is described by an approximately hyperbolic curve with a K, of 1.7 mM ± 0.03 and a Vmax of 0.42 ± 0.02 μmol of ATP hydrolyzed/mg/min. Some fractionation of the Mg2+-ATPase activity could be achieved by sedimentation of the microsomal membrane in a sucrose density gradient as shown in Fig. 2. The Mg2+-ATPase activity was associated with a membrane protein peak of buoyant density 1.22 g/ml which is similar to the density observed for the plasma membrane of S. cerevisiae by others (26, 27).

Comparison with Other Known ATPases—Fig. 3 shows the pH dependence of the microsomal membrane Mg2+-ATPase activity in the presence and absence of oligomycin, an inhibitor of the mitochondrial ATPase. At the pH optimum of 5.5, over 60% of the ATPase activity is oligomycin-resistant and can be attributed to the plasma membrane Mg2+-ATPase. The yeast mitochondrial Mg2+-ATPase has a pH optimum near 9 (9) and is sensitive to oligomycin. Therefore, the oligomycin-sensitive ATPase activity at pH 9.0 to 9.5 is probably caused by contamination of the microsomal preparation by the mitochondrial enzyme. Also, the pH 9.0 to 9.5 activity is completely sensitive to the mitochondrial ATPase inhibitors efrapeptin and Dio 9 (28), while the pH 5.5 activity is only partially sensitive to these inhibitors (data not shown).

Since the metal ion pumps of the mammalian plasma membrane enzyme are stimulated by certain cations, the yeast

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1 The abbreviations used are: SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride.
The Yeast Plasma Membrane Mg²⁺-ATPase

Vanadate, a known inhibitor of plasma membrane ATPases (29-31), inhibits the pH 5.5 activity of the enzyme (Fig. 4) without affecting the pH 9.5 activity (data not shown). The $K_i$ for vanadate is approximately 11 µM, which is similar to its $K_i$ for the Na⁺ activity of the (Na⁺-K⁺) ATPase (30) and the Neurospora Mg²⁺-ATPase (29). In this experiment, inhibition by vanadate was never greater than 85%, probably due to contamination of the plasma membrane ATPase by the mitochondrial vanadate-resistant Mg²⁺-ATPase. Since vanadate has been proposed as a transition state analogue for protein phosphate hydrolysis (33), vanadate inhibition of the yeast enzyme suggests that a protein phosphate intermediate is involved in the enzymatic mechanism.

Demonstration of a Stable Phosphorylated Intermediate in the Yeast Plasma Membrane

The existence of phosphorylated intermediates associated with the yeast plasma mem-

plasma membrane enzyme was assayed in the presence of exogenously added cations. The yeast enzyme is not affected by Ca²⁺ (5 to 500 µM) and it is slightly inhibited by Na⁺ (64% inhibited by 375 mM) and K⁺ (28% inhibited by 50 mM). Experiments using similar concentrations of Tris showed that this inhibition appears to be due to increasing ionic strength. Strophanthidin, a specific inhibitor of the (Na⁺-K⁺)-ATPase did not inhibit the yeast enzyme. Therefore, the yeast enzyme does not have the properties of the mammalian plasma membrane metal ion pumps.

Fig. 2. Density of the microsomal membrane fraction ATPase: protein determination; ---, ATPase activity. Ten microliters of each fraction was assayed in a medium containing 50 mM Tris/succinate, pH 5.5, 5 mM MgCl₂, and 3.6 mM Tris/[γ-³²P]ATP. The density indicated on the bottom scale was determined by refractometry for each fraction.

Fig. 3. pH profile and oligomycin sensitivity of the ATPase activity. Each reaction mixture contained 50 mM Tris/succinate, pH 5.5, 5 mM MgCl₂, 15 µg of protein, and 3.6 mM Tris/[γ-³²P]ATP. Each time point represents the average of two separate determinations. ○, assayed in the absence of oligomycin; ●, assayed in the presence of oligomycin (133 µg of oligomycin/mg of protein.)

Fig. 4. Inhibition of the plasma membrane Mg²⁺-ATPase by vanadate. The reaction mixtures contained 50 mM Tris/succinate, pH 5.5, 3.6 mM [γ-³²P]ATP, 5 mM MgCl₂ 15 µg of protein, and the amount of sodium vanadate indicated in the figure. Every point represents the average of two separate determinations.

Fig. 5. The formation of phosphorylated intermediates at pH 5.5. The phosphorylation reaction contained 50 mM Tris/succinate, pH 5.5, 22 µM [γ-³²P]ATP (specific activity, 3 Cl/mmoll), and 375 µg of protein. After incubation at 23°C, the reaction was stopped by the addition of cold trichloroacetic acid. The washed trichloroacetic acid precipitates were electrophoresed on low pH SDS-polyacrylamine slab gels. Denatometer scans were made from an autoradiogram of the dried gel. See "Experimental Procedures" for a detailed description. A, no additions, 10-s incubation. B, MgCl₂ to 5 mM was added before the 10-s incubation. C, MgCl₂ to 5 mM was added before the incubation was started. After 10 s, unlabeled ATP to 10 mM was added and the incubation continued for another 10 s.
The Yeast Plasma Membrane Mg²⁺-ATPase

A. -Mg²⁺

0. +Mg²⁺ I

C. +Mg²⁺ +Vanadate

123456789

CM From top of gel

FIG. 6. Inhibition of phosphorylation by vanadate at pH 5.5. The reaction mixture contained 50 μM Tris/succinate, pH 5.5, 20 μM [γ-3²P]ATP (specific activity, 2 Ci/mmol), and 375 μg of protein. Phosphorylation, electrophoresis, and autoradiography were carried out as described in the legend to Fig. 5. A, no additions. B, MgCl₂ added to 5 mM before the incubation. C, MgCl₂ to 5 mM and Na₃VO₄ to 1.6 mM were added before the incubation.

5.5 three peaks labeled with phosphate appear quickly, show Mg²⁺-stimulated formation, and disappear after exposure of the enzyme to excess unlabeled ATP. The molecular weights of these phosphoproteins (see "Experimental Procedures") were found to be 210,000 for protein in peak A, 160,000 for peak B protein, and 115,000 for peak C protein. The phosphate present in peaks A and B in the absence of Mg²⁺ could be due to the presence of exogenous Mg²⁺ in the membrane fraction.

FIG. 7. Hydroxylamine sensitivity of the phosphorylated intermediates at pH 5.5. The phosphorylation reaction contained 50 mM Tris/succinate, pH 5.5, 22 μM [γ-3²P]ATP (specific activity, 3 Ci/mmol), and 375 μg of protein. The phosphorylation, electrophoresis, and autoradiography were done as described in the legend to Fig. 5. The following additions were made to the phosphorylation reaction: A, no additions, 5-s incubation. B, MgCl₂ was added to 5 mM, 5-s incubation. C, MgCl₂ was added to 5 mM. After 5 s, 10 μM unlabeled Tris/ATP was added to the reaction. After 15 s, the reaction was terminated by the addition of cold trichloroacetic acid. D, MgCl₂ was added to 5 mM. The reaction was terminated with cold trichloroacetic acid before the addition of enzyme. E, A sample of [γ-3²P]ATP alone (20,000 cpm).

TABLE I

| Time for phosphorylation incubation (s) | Length of chase (s) | Phosphate bound/mg of protein (pmol) | Phosphate remaining (pmol) | Phosphate remaining (%) |
|---------------------------------------|--------------------|-------------------------------------|----------------------------|------------------------|
| s                                     | s                  | %                                   | %                          |                        |
| 15                                    | 0                  | 0.29                                | 100                        | 0.43                   | 100                      |
| 10°                                   | 0                  | 0.31                                | 106                        | 0.41                   | 95                       |
| 15°                                   | 3                  | 0.07                                | 20                         | 0.07                   | 16                       |
| 15°                                   | 7                  | 0.09                                | 29                         | 0.09                   | 22                       |
| 15°                                   | 11                 | 0.12                                | 37                         | 0.11                   | 96                       |
| 15°                                   | 15                 | 0.07                                | 21                         | 0.04                   | 12                       |

All these samples came from the same incubation mixture.
while that present after ATP chase might be caused by an occluded site on the protein due to the high concentration of crude membrane required for the phosphorylation. Both of the previous phenomena could also be explained by the action of another type of phosphorylation system or the presence of more than one enzyme in each peak.

Vanadate, an inhibitor of the yeast plasma membrane Mg²⁺-ATPase (Fig. 4), also inhibits the phosphorylation of the phosphoproteins found in peaks A and C. In addition, Fig. 6 shows that the phosphorylation of peak B is relatively resistant to vanadate. For the phosphorylation experiment, the concentration of vanadate was 1.6 mM, a concentration which inhibited 96% of the hydrolytic activity. At the high protein concentration used for the assay, micromolar concentrations of vanadate did not inhibit hydrolysis (see "Discussion").

Incubation of the labeled enzyme with hydroxylamine, which breaks down the aspartate-P, linkage of the Ca²⁺-ATPase (34) and the (Na⁺-K⁺)-ATPase (35, 36), also causes the dephosphorylation of the phosphoprotein associated with the yeast plasma membrane Mg²⁺-ATPase. Incubation of the phosphoprotein in the acetic buffer used for hydroxylamine treatment did not cause the disappearance of any peaks (Fig. 7C), while incubation in this buffer in the presence of hydroxylamine caused the dephosphorylation of the intermediates present in all the peaks (Fig. 7D). Also, the presence of hydroxylamine in the reaction mixture completely inhibited the hydrolysis activity of the enzyme (data not shown).

The phosphorylation reaction associated with the yeast enzyme, like the hydrolysis reaction, appears to be pH-dependent. Fig. 8 shows that at pH 7.0 very little of peak C is formed and only the formation of peak A appears to be Mg²⁺-dependent. The proteins in both peaks A and B, however, rapidly dephosphorylate in the presence of excess unlabeled ATP (Fig. 8C). Control experiments show that no label can be seen in the region of peaks A and B in the absence of enzymatic activity (Fig. 8D) and that the large peak with a mobility of 0.7 is free ATP (Fig. 8E) as observed by other workers.

Due to the low background at pH 7.0, the time course of dephosphorylation was examined in detail at this pH. Fig. 8 shows that at 25°C the phosphate bound in peaks A and B could be chased by a 10-s pulse of excess unlabeled ATP. In order to examine the dephosphorylation more closely, the enzymatic reactions were slowed by incubation of the phosphorylation and dephosphorylation reactions at 0°C. As is shown in Table 1, at the earliest time point, 3 s after the addition of unlabeled ATP, only 16 to 20% of the label remains bound to the protein. The results show that dephosphorylation is as fast as the turnover time for ATP hydrolysis, and they suggest that a phosphorylated intermediate is a possible intermediate in the mechanism of hydrolysis (see "Discussion").

DISCUSSION

The ATPase of the plasma membrane of the yeast, S. cerevisiae, has been characterized with regard to its sensitivity to inhibitors, pH dependence, response to added cations, and sedimentation properties. The enzyme was shown to be Mg²⁺-dependent, oligomycin-resistant, have a pH optimum of 5.5, a maximum velocity of 0.42 μmol of ATP hydrolyzed/mg/min, and a Kₘ of 1.7 mM. The buoyant density of the membrane fraction associated with the ATPase activity is 1.22 g/ml. The enzyme is not stimulated by exogenously added cations and is resistant to strophantinidin and sensitive to vanadate.

The activity of this plasma membrane Mg²⁺-ATPase is associated with the Mg²⁺-dependent formation of at least three stable phosphoproteins. These proteins of Mᵦ, 210,000, 160,000, and 115,000 show rapid Mg²⁺-stimulated phosphorylation, dephosphorylate rapidly in the presence of excess unlabeled ATP, and exhibit kinetics of phosphorylation and dephosphorylation which are compatible with their involvement as intermediates in the hydrolysis reaction. The formation of two phosphoprotein peaks, A and C, is inhibited by vanadate, while the stability of all three phosphoprotein peaks is sensitive to hydroxylamine.

These preliminary studies on the activity of the microsomal Mg²⁺-ATPase have shown the enzyme to be distinct from both the mitochondrial Mg²⁺-ATPase, and the well characterized metal ion pumps of the mammalian plasma membrane. The enzyme shows a pH optimum of 5.5 characteristic of the yeast plasma membrane ATPase and well removed from the pH optimum of 9 exhibited by the mitochondrial F₁ ATPase in yeast (9). Also, the bouyant density of the ATPase associated membrane fraction is 1.22 g/ml, within the range reported for the yeast plasma membrane (26, 27), and sufficiently different from the bouyant density of 1.16 to 1.18 reported for the yeast mitochondrial membrane (26). The yeast plasma membrane enzyme also appears to be resistant to oligomycin and only partially sensitive to the mitochondrial inhibitors, efrapeptin and Dio 9 (28). In addition, the yeast enzyme is not stimulated by any other ions that stimulate the mammalian metal ion pumps, nor is it inhibited by strophantinidin, a specific inhibitor of the (Na⁺-K⁺)-ATPase.

Vanadate has recently been shown to inhibit plasma membrane ion pumps; in fact, the (Na⁺-K⁺)-ATPase of both the red blood cell (31) and the kidney (30), the Mg²⁺-ATPase of Neurospora (29) and the Ca²⁺-ATPase of the red blood cell (32) are all vanadate-sensitive. Interestingly, vanadate seems to inhibit only those ATPases in which a phosphorylated intermediate is formed during turnover (29-32, 37) and it appears to bind as a transition state analogue at the site where phosphate is released (30, 38). The results presented here show that vanadate inhibits the yeast enzyme and would suggest that the Mg²⁺-ATPase described here also functions by formation of a phosphorylated intermediate.

At least three rapidly forming, Mg²⁺-sensitive phosphoproteins, which phosphorylate and dephosphorylate rapidly enough to be intermediates, were found in the yeast plasma membrane. The phosphorylation of two phosphoprotein peaks, A and C, was inhibited by vanadate, suggesting that one or both of these may be intermediates in the ATPase activity. All three of the phosphoprotein peaks formed at pH 5.5 are sensitive to inhibition in the presence of hydroxylamine (which dephosphorylates the phosphorylated intermediates of the Ca²⁺-ATPase (34) and the (Na⁺-K⁺)-ATPase (35, 36)), and hydroxylamine also inhibits the hydrolysis of the yeast plasma membrane enzyme. Taken together, these results suggest, but do not prove, that peaks A or C, or both, may be phosphorylated intermediates in the activity of the Mg²⁺-ATPase of the yeast plasma membrane.

The view that the phosphoproteins found in the yeast plasma membrane are intermediates in the Mg²⁺-ATPase reaction must be taken with caution because the amount of phosphoprotein found is low, the phosphorylated intermediates cannot be directly linked to the ATPase reaction, and all the phosphoproteins do not fit the proposed role as intermediates.

The quantity of phosphorylated intermediate is low compared to that found for the other plasma membrane ion pumps. If the amount of bound phosphate is stoichiometric with the number of copies of the enzyme, the estimated turnover number is 1500 to 2000 s⁻¹ (using a specific activity of 0.2 μmol of ATP hydrolyzed/mg/min, and between 0.5 and 2 The phosphorylation of the (Na⁺-K⁺)-ATPase was also inhibited by vanadate (unpublished observation).
1.5 pmol of phosphate bound/mg of protein). This is considerably higher than the turnover of 100 to 500 s\(^{-1}\) (39) for the metal ion pumps of the eukaryotic plasma membrane. This may be due to the fact that the dephosphorylation is not the rate-limiting step, the intermediates are not stable during isolation, or that the formation of the phosphoryprotein is reduced due to the high amount of protein required for the assay.

A direct relationship between the phosphoryproteins and ATP hydrolysis has not been demonstrated. The use of a more purified enzyme preparation would allow one to resolve the ambiguities resulting from the use of a crude system (i.e., the incomplete dephosphorylation of the phosphoryproteins seen in Fig. 5, the Mg\(^{2+}\) independence of the phosphorylation reaction for peak B protein seen in Fig. 8, and the high concentration of vanadate required to inhibit phosphorylation), and to definitely associate the phosphoryprotein(s) with the ATPase hydrolytic activity. For example, the Mg\(^{2+}\) independence of the protein in peak B and its resistance to vanadate make it unlikely that peak B is a phosphorylated intermediate associated with the yeast plasma membrane enzyme.

The high concentration of vanadate required for the inhibition of hydrolysis in the phosphorylation assay may be indicative of the tendency of vanadate to bind to itself and to proteins. Vanadate has been shown to form oligomers with itself at low pH, to bind to proteins such as hemoglobin, and to be reduced to an inactive form under certain physiological conditions (31). Considering the large amounts of crude membrane fraction in the phosphorylation assay, the high amount of vanadate may be necessary to maintain sufficient quantities of free vanadate in the reaction mixture.

The functional significance of the Mg\(^{2+}\)-dependent plasma membrane ATPase has not been established, but it may be involved in cellular transport. The transport of amino acids, sugars, and other small metabolites in various fungi has been shown to involve proton gradients. In *Neurospora*, an ATP-dependent electrogenic proton pump has been characterized and shown to be involved in the plasma membrane transport system (10–12). In the yeast *S. cerevisiae*, intracellular ATP is required for active cellular uptake of small metabolites, and a plasma membrane Mg\(^{2+}\)-ATPase has been characterized (9, 40). Using the yeasts *S. cerevisiae* and *S. carlsbergensis*, Seaston and various associates have demonstrated the concurrent uptake of protons and various sugars and amino acids (41). In addition, amino acid accumulation in ATP-starved *S. cerevisiae* can be driven by the spontaneous influx of H\(^+\) ions coupled to K\(^+\) efflux (42).

This study and other papers have described a Mg\(^{2+}\)-dependent ATPase associated with the plasma membrane in *S. cerevisiae*. This result and the involvement of proton gradients in cellular transport processes suggests the hypothesis that the yeast plasma membrane Mg\(^{2+}\)-ATPase is an ion pump involved in the maintenance of the proton gradient necessary for active transport to occur in this organism. Although this hypothesis is attractive, a direct connection between ATPase activity and proton translocation has not been demonstrated in yeast.

It is useful to compare the properties of the yeast Mg\(^{2+}\)-ATPase with other ATP cation pumps. These pumps fall into two broad classes, those that pump metal cations and those that pump protons. The metal cation ATPases have been shown to have phosphorylated intermediates involved in their activity, while no phosphorylated intermediate has been detected for the proton pumps. Recently, several ATPases have been observed and partially characterized which do not completely fit into either of these classes. The (H\(^+\)-K\(^+\))-ATPase of the gastric mucosa (43, 44) and the *Neurospora* Mg\(^{2+}\)-ATPase (11, 12) are present in the eukaryotic plasma membrane and pump protons. It is of interest to compare these pumps with the two previous classes. The gastric mucosa pump has been shown to involve a phosphorylated intermediate, while the *Neurospora* enzyme is vanadate-sensitive; implying a mechanism similar to the metal ion pumps of the mammalian plasma membrane. Results in this paper indicate that the yeast enzyme (a putative proton pump) is also inhibited by vanadate and may involve a vanadate-sensitive phosphorylated intermediate, suggesting that the yeast enzyme resembles the gastric mucosa and *Neurospora* enzyme and that all of these ATPases are similar to the metal ion pumps of the mammalian plasma membrane.

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REFERENCES

1. Futai, M., Sternweis, P. C., and Heppel, L. A. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 2725–2729
2. McCarty, R. E. (1978) Curr. Top. Bioenerg. 7, 245–278
3. Pedersen, P. L. (1975) Bioenergetics 6, 245–275
4. Jorgensen, F. L. (1975) Q. Rev. Biophys. 7, 239–274
5. MacLennan, D. H., and Holland, P. V. (1975) Annu. Rev. Biochem. Bioenerg. 4, 377–404
6. Nurminen, T., Oura, E., and Suomalainen, H. (1970) Biochem. J. 116, 61–69
7. Matile, Ph., Moor, H., and Muhlethaler (1967) Arch. Mikrobiol. 58, 201–211
8. Cartledge, T. G., and Lloyd, D. (1973) Biochem. J. 132, 609–621
9. Delhez, J., Dufour J. P., Thines, D., and Goffeau, A. (1977) Eur. J. Biochem. 79, 319–328
10. Bowman, B. J., and Slayman, C. W. (1977) J. Biol. Chem. 252, 3357–3363
11. Slayman, C. L., Long, W. S., and Lu, C. Y.-H. (1973) J. Membr. Biol. 14, 305–308
12. Scarborough, G. A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1485–1488
13. Sherman, F., Fink, G. R., and Lawrence, C. W. (1977) Methods in Yeast Genetics, p. 61, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.
14. Duell, E. A., Inoue, S., and Utter, M. F. (1964) J. Biol. Chem. 239, 1762–1773
15. Wiley, W. R. (1974) Methods Enzymol. 31A, 609–626
16. Schatz, G., and Kovač, L. (1974) Methods Enzymol. 31A, 627–632
17. Bailey, J. L. (1967) Techniques in Protein Chemistry, 2nd Ed., pp. 340–346, Elsevier Publishing Co., New York
18. Goldin, S. M. (1977) J. Biol. Chem. 252, 5630–5642
19. Glynn, I. M., and Chappell, J. R. (1964) Biochem. J. 90, 147–149
20. Blostein, R. (1968) J. Biol. Chem. 243, 1957–1965
21. Drickamer, L. K. (1975) J. Biol. Chem. 250, 1952–1954
22. Inesi, G., Maring, E., Murphy, A. J., and McFarland, B. H. (1970) Arch. Biochem. Biophys. 138, 285–294
23. Fairbanks, G., and Abruč, I. (1972) J. Supramol. Struct. 1, 66–75
24. Åhrv, J., and Fairbanks, G. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1216–1220
25. Studier, F. W. (1973) J. Mol. Biol. 79, 237–248
26. Schatz, G., Tuppy, H., and Klima, J. (1963) Z. Naturforsch. Teil B 18, 145–153
27. Furhman, G. F., Wehrli, E., and Boehm, C. (1974) Biochim. Biophys. Acta 363, 295–310
28. Lardy, H., Reed, P., and Lin, C. C. (1975) Fed. Proc. 34, 1707–1710
29. Bowman, B. J., Mainzer, S. E., Allen, K. E., and Slayman, C. W. (1978) Biochim. Biophys. Acta 512, 13–28
30. Cantley, L. C., Jr., Cantley, L. G., and Josephson, L. (1978) J. Biol. Chem. 255, 7361–7368
31. Cantley, L. C., Jr., Resh, M., and Guidotti, G. (1978) Nature
3.32 The Yeast Plasma Membrane Mg\(^{2+}\)-ATPase

(1978) Fed. Proc. 37, 313

32. Bond, G. H., and Hudgins, P. M. (1978) J. Biol. Chem. 248, 8222-8226

33. Post, R. L., and Kume, S. (1973) J. Biol. Chem. 248, 6993-7000

34. Nishigaki, I., Chen, F. T., and Hokin, L. E. (1974) J. Biol. Chem. 249, 4911-4916

35. Lopez, V., Stevens, T., and Lindquist, R. L. (1976) Arch. Biochem. Biophys. 175, 1-38

36. Van Etten, R. L., Waymack, P. P., and Rehkop, D. M. (1974) J. Am. Chem. Soc. 96, 6782-6785

37. Josephson, L., and Cantley, L. C., Jr. (1977) Biochemistry 16, 4572-4578

38. Dufour, J.-P., and Goffeau, A. (1978) J. Biol. Chem. 253, 7026-7032

39. Seaston, A., Inkson, C., and Eddy, A. A. (1978) Biochem. J. 134, 1031-1043

40. Eddy, A. A., Backen K., and Watson G. (1970) Biochem. J. 120, 853-858

41. Sachs, G., Spenney, J. G., and Lewin, M. (1978) Physiol. Rev. 58, 106-173

42. Sachs, G., Chang, H. H., Rabon, E., Schackman, R., Lewin, M., and Saccamani, G. (1976) J. Biol. Chem. 251, 7890-7898
Characterization of the plasma membrane Mg2+-ATPase from the yeast, Saccharomyces cerevisiae.
G R Willsky

J. Biol. Chem. 1979, 254:3326-3332.

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