H\(^+\)-translocating, Mg\(^{2+}\)-ATPase was solubilized from vacuolar membranes of *Saccharomyces cerevisiae* with the zwitterionic detergent N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate and purified by glycerol density gradient centrifugation. Partially purified vacuolar membrane H\(^+\)-ATPase, which had a specific activity of 18 units/mg of protein, was separated almost completely from acid phosphatase and alkaline phosphatase. The purified enzyme required phospholipids for maximal activity and hydrolyzed ATP, GTP, UTP, and CTP, with this order of preference. Its \(K_m\) value for Mg\(^{2+}\)-ATP was determined to be 0.21 mM and its optimal pH was 6.9. ADP inhibited the enzyme activity competitively, with a \(K_i\) value of 0.31 mM. The activity of purified ATPase was strongly inhibited by \(N,N'\)-dicyclohexylcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, tributyltin, 7-chloro-4-nitrobenzoxazole, diethylstilbestrol, and quercetin, but was not affected by oligomycin, sodium azide, sodium vanadate, or miconazole. It was not inhibited at all by antiserum against mitochondrial F\(_1\)-ATPase or mitochondrial F\(_1\)-ATPase inhibitor protein. These results indicated that vacuolar membrane H\(^+\)-ATPase is different from either yeast plasma membrane H\(^+\)-ATPase or mitochondrial F\(_1\)-ATPase. The vacuolar membrane H\(^+\)-ATPase was found to be composed of two major polypeptides \(a\) and \(b\) of \(M_c = 89,000\) and 64,000, respectively, and a \(N,N'\)-dicyclohexylcarbodiimide binding polypeptide \(c\) of \(M_c = 19,500\), whose polypeptide composition was also different from those of either plasma membrane H\(^+\)-ATPase or mitochondrial F\(_1\)-ATPase of *S. cerevisiae*.

Previous studies in our laboratory have demonstrated the existence in the vacuolar membrane of the yeast *Saccharomyces cerevisiae* of an H\(^+\)-translocating ATPase (1) and several active transport systems, which are specific for Ca\(^{2+}\) (2), arginine (3), and nine other amino acids (4). Using a preparation of right-side-out vacuolar membrane vesicles of high purity, we showed that the H\(^+\)-ATPase generates an electrochemical potential difference of protons across the membrane of 180 mV, interior acid (1), and that all the above active transport systems are catalyzed by a mechanism of H\(^+\)/substrate antiport that is driven by the electrochemical potential difference of protons produced upon hydrolysis of ATP by the H\(^+\)-ATPase (2-4). More recently, we found that the composition and amounts of amino acid pools in the vacular compartment change dynamically with change of the nitrogen source for growth, strongly suggesting that the vacuoles are in fact regulating the nitrogen metabolism and cellular growth by ensuring homeostasis of amino acids in the cytoplasm (5). Incidentally, these findings provided new insight into the role(s) of vacuoles in cellular metabolic processes and prompted us to make genetic studies on growth regulation by the Ca\(^{2+}\) ion (6).

This paper reports the purification and properties of the vacuolar membrane H\(^+\)-ATPase from *S. cerevisiae*. A partially purified enzyme, which was free from mitochondrial F\(_1\)-ATPase (7) and plasma membrane H\(^+\)-ATPase (8-10), was found to have unique enzymological and protein-chemical properties, differing strikingly from those of plasma membrane H\(^+\)-ATPase and mitochondrial F\(_1\)-ATPase.

**MATERIALS AND METHODS**

**Chemicals, Antiserum, and Mitochondrial F\(_1\)-ATPase Inhibitor Protein**

The zwitterionic detergent ZW3-14 was obtained from Calbiochem Behring, Mes, EDAC, and PMSF were from Sigma. Soybean phospholipids were obtained from Sigma and prepared as described by Kagawa and Racker (11). ATP and other nucleotides were from Boehringer Mannheim. DES was from Nakarai Chemicals, Tokyo. DCCD was from the Protein Research Foundation, Osaka. \([\gamma^3\text{P}]\) ATP (3,000 Ci/mmol) and \([\beta^3\text{P}]\)DCCD (50 mCi/mmol) were purchased from Amersham. The other chemicals used were commercial products of analytical grade and were described in previous papers (1-3). Antiserum against mitochondrial F\(_1\)-ATPase and purified mitochondrial F\(_1\)-ATPase inhibitor protein (12) were generous gifts from Dr. K. Tagawa, Osaka University, Osaka. Miconazole was a gift from Dr H. Yamauchi, Teikyo University, Tokyo.

**Strain and Culture Conditions**

The haploid strain of *Saccharomyces cerevisiae* X2180-1A, from the Yeast Genetic Stock Center, Berkeley, was used throughout. For isolation of vacuolar membrane vesicles and plasma membranes on a large scale, cells were grown aerobically in YEPD medium containing 1% yeast extract (Difco), 2% peptone, and 2% glucose at 30 °C in a 10-liter Magneferm fermentor (New Brunswick Scientific Co. Inc.) that was aerated at an air flow of 6 liters/min and agitated at 200 rpm. Exponentially growing cells (4 × 10\(^7\) cells/ml) were harvested.
by centrifugation as described previously (3).

For isolation of submitochondrial particles, cells were grown aerobically at 30 °C in yeast extract-salts medium (13) supplemented with 2% ethanol as a carbon source and harvested in the middle of the exponential phase of growth.

### Assays of H^+-ATPases and Other Enzymes

Vacuolar membrane H^+-ATPase was assayed at 30 °C in a final volume of 0.1 ml containing 25 mM Mes/Tris (pH 6.9), 5 mM ATP-2Na, 5 mM MgCl₂, and enzyme. For assay of the enzyme after solubilization, sonicated soybean phospholipids were added to the assay mixture at a final concentration of 0.1 mg/ml. When indicated, the inhibitor in ethanol solution (DCCD, oligomycin, DES, quercetin, tributyltin, and NBD-CI) or dimethyl sulfoxide solution (miconazole) was added to the assay mixture at a final concentration of solvent of less than 0.2% (v/v). The reaction was started by the addition of Mg^2+ATP and, after 5–20 min of incubation, was stopped by adding 0.1 ml of 5% (w/v) SDS. Inorganic phosphate liberated was measured by the method of Omhishi (14) and 1 unit of enzyme was defined as the amount liberating 1 µmol of inorganic phosphate per min under the standard conditions described above. For determination of the Kᵦ value, the activity of H^+-ATPase was assayed by measuring the release of ^[^32P]P from [γ-[^32P]]ATP (1–5 Ci/mol) in the standard mixture as described above. After 1 min at 30 °C, the reaction was stopped by adding 0.061 ml of 13.2% trichloroacetic acid, 1 mM phosphoric acid, and 10 mM ATP, and then 0.3 ml of 4% perchloric acid (15). Inorganic phosphate liberated was extracted as phosphomolybdcic acid with 4 ml of water-saturated isobutyl alcohol/benzen and, after centrifugation at 4,500 × g for 10 min, the radioactivity in the organic phase (2 µl) was counted as Cerenkov radiation in a Beckman LS-9000 scintillation counter (16).

The standard reaction mixtures for assays of plasma membrane H^+-ATPase and mitochondrial F₁-ATPase were similar to that for assay of vacuolar membrane H^+-ATPase, except for the pH of the medium. The pH of the reaction mixture for assay of plasma membrane H^+-ATPase was pH 5.5, obtained with 25 mM Mes/Tris buffer, and that for assay of mitochondrial F₁-ATPase was pH 9.0, obtained with 25 mM Tris/HC1 buffer.

Acid phosphatase (17), alkaline phosphatase (17), and o-mannosidase (18) were assayed by published methods. One unit of enzyme activity was defined as the amount catalyzing hydrolysis of 1 µmol of substrate per min under standard assay conditions.

### Purification of Vacuolar Membrane H^+-ATPase from S. cerevisiae

**Purification of Vacuolar Membrane H^+-ATPase**

**Step 1: Vacuolar Membrane Vesicles**—The procedure used previously for preparing pure vacuolar membrane vesicles (3) was modified for use on a large scale. Cell preparations grown on 4 × 10^6 cells/ml were harvested by centrifugation at 4500 × g for 3 min, washed twice with distilled water at room temperature, and suspended in 1 mM sorbitol at a density of 2 × 10^10 cells/ml. To this suspension, zymolyase was added to a final concentration of 1 unit/ml, and the mixture was incubated at 30 °C for 30 min with gentle shaking. This treatment converted more than 95% of the cells to spheroplasts, which were then recovered by centrifugation and washed twice with 1 mM sorbitol with centrifugation (2200 × g for 5 min at 4 °C). The white layer on the top of the tubes, which contained most of the vacuoles, was collected and resuspended in Buffer A with a homogenizer. Then, 15 ml of Buffer B (10 mM Mes/Tris (pH 6.9), 0.5 mM MgCl₂, 8% Ficoll-400) was layered on top. After recentrifugation under the conditions described above, the vacuoles were recovered from the top of the tubes almost free from contaminating lipid granules and other membranous organelles. The vacuoles thus obtained were converted to vacuolar membrane vesicles by diluting them first with an equal volume of double concentration Buffer C (10 mM Mes/Tris (pH 6.9), 5 mM MgCl₂, 25 mM KC1) and then with 2 volumes of Buffer C. The vesicles were recovered by centrifugation (37,000 × g, 20 min).

**Step 2: EDTA Wash**—Vacuolar membrane vesicles prepared as described above (approximately 30 mg of protein) were suspended in a solution of 10 mM Tris/HCl (pH 7.5), 1 mM EDTA at a concentration of protein of 1 mg/ml and homogenized by 5 strokes of a Dounce homogenizer. Unless otherwise noted, all preparations were carried out at 0–4 °C. This EDTA wash was repeated 3 times with recovery of 70% of the total protein and no loss of ATPase activity.

**Step 3: Solubilization**—The EDTA-washed membranes were suspended in a protein-free solution of 0.1 M sorbitol with centrifugation (2200 × g, 20 min). The pellet obtained was solubilized in 1 ml of 10 mM Tris/HCl (pH 7.5), 1 mM EDTA, 2 mM DTT, 0.5 mM PMSF, and 0.005% (w/v) ZW3-14 was added dropwise under vigorous stirring to a final weight ratio of detergent to protein of 1.0. The mixture was kept at 4 °C for 15 min with gentle shaking and then centrifuged at 108,500 × g for 60 min in a Hitachi RP65 rotor. The supernatant was collected as the solubilized fraction.

**Step 4: Glyceraldehyde-3-P Dehydrogenase Activity**—Aliquots (0.25 ml) of the supernatant obtained at Step 3 were layered on top of an 8-ml linear gradient of 20–50% (v/v) glycerol in a solution of 10 mM Tris/HCl (pH 7.5), 1 mM EDTA, 2 mM DTT, 0.5 mM PMSF, and 0.005% (w/v) ZW3-14, and centrifuged at 180,000 × g for 8 h in a Hitachi RPS5 rotor at 4 °C. Then fractions (0.5 ml each) were collected from the bottom of the tubes using a Ferista mini pump (ATTO Co.). Fraction I shows the distribution profiles of protein and activities of H^+-ATPase, o-mannosidase, acid phosphatase, and alkaline phosphatase. Under the conditions used, H^+-ATPase sedimented faster than the bulk of the protein and was well separated from acid phosphatase and alkaline phosphatase. The peak fraction had a specific activity of 16 units/mg of protein. Fractions with activity of more than 16 units/mg of protein were pooled and used as purified vacuolar membrane H^+-ATPase.

**Results**

**Purification**—Table I summarizes the purification. More than 35-fold purification was attained with recovery of 5% of
The specific activity of H+-ATPase was increased about 7-fold at this step with elimination of 30% of the total protein (Table I): this increase in specific activity may also result from elimination of some inhibitory divalent cations such as Cu2+ and Zn2+ ions (1) from the membranes.

We tested solubilizing effects of several detergents and found that the zwitterionic detergent ZW3-14 was the best; detergents such as Triton X-100, cholic acid, Sarkosyl, and Tween 80 were not effective. We also found that the weight ratio of the detergent to protein was critical for solubilization. In Step 3, in which the protein concentration of EDTA-washed membranes was adjusted at 5 mg/ml, 10 mM ZW3-14 solubilized 30% of H+-ATPase activity and 50% of the protein from vacuolar membranes. At a concentration of 7 mM or less, the detergent was not effective for solubilization, while at 16 mM it solubilized more than 70% of the membrane protein, but the solubilized ATPase activity was rapidly inactivated. Finally, we found that at the optimal concentration of ZW3-14 of 14 mM, or a weight ratio of the detergent to protein of 1.0, approximately 70% of the membrane protein and a maximum of 55% of the H+-ATPase activity were recovered in the supernatant fraction (Table I).

We tried to purify the enzyme in the supernatant by several conventional techniques. No appreciable success was obtained by column chromatography through DE 52 (Whatman) or DEAE-Sepharose (Pharmacia), gel filtration with high performance liquid chromatography (SW-3000, Toyo Soda), affinity chromatography with Blue Sepharose CL-6B (Pharmacia), or isoelectric focusing electrophoresis. Glycerol density gradient centrifugation was the only effective procedure.

The concentration of the detergent ZW3-14 in the glycerol density gradient was found to be critical for recovery and separation of the enzyme activity. At a concentration of 0.015% or more, the recovery was less than 7%. Addition of KCl (25-300 mM) to the gradient also reduced the recovery. Under the conditions described for Step 4, approximately 68% of the ATPase activity loaded on the gradient was recovered.

Properties of the Purified Vacuolar Membrane H+-ATPase—The purified vacuolar membrane H+-ATPase required phospholipids for maximal activity (Fig. 2). The activity was stimulated 3-fold by phospholipids at more than 0.1 mg/ml. Under the standard assay conditions in the presence of phospholipids, the K0.5 value for ATP was determined to be 0.21 mM. ADP inhibited the enzyme activity competitively.
and its $K_i$ value was determined to be 0.31 mM. The purified enzyme hydrolyzed ATP, GTP, UTP, and CTP, with this order of preference (Table II). ADP, AMP, and pNPP were not hydrolyzed. The optimal pH was determined to be 6.9.

The effects of antiserum against mitochondrial F$_1$-ATPase and the mitochondrial F$_1$-ATPase inhibitor protein on the activity of the purified enzyme were examined. The activity was not inhibited at all by the serum, which inhibited the F$_1$-ATPase activity in submitochondrial particles completely (Fig. 3A). Fig. 3B shows that the activity of the purified vacuolar membrane H$^+$.ATPase was not affected by the mitochondrial F$_1$-ATPase inhibitor protein. These results clearly indicate that the vacuolar membrane H$^+$.ATPase is a unique ATPase and differs from mitochondrial F$_1$-ATPase. We found that the purified vacuolar membrane H$^+$.ATPase is not cold-labile.

DCCD is a potent inhibitor of various H$^+$.translocating ATPases (Fig. 4A). The $K_i$ values for DCCD of the purified vacuolar enzyme and ATPases in vacuolar membrane vesicles, submitochondrial particles, and plasma membranes were determined to be 0.8, 2.0, and 8.0 $\mu$M, respectively. Thus, the vacuolar membrane ATPase was approximately 10-fold less sensitive to DCCD than mitochondrial F$_1$-ATPase and 4-fold more sensitive than plasma membrane ATPase (Fig. 4A). The

**TABLE II**

<table>
<thead>
<tr>
<th>Substrate specificity of purified H$^+$.ATPase</th>
<th>Substrate</th>
<th>Relative activity$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>GTP</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>CTP</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>pNPP</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

$^*$Activity was measured with 1 mM substrate as indicated under standard conditions.

**Fig. 3.** Effects of antiserum against F$_1$-ATPase (A) and mitochondrial F$_1$-ATPase inhibitor protein (B) on purified vacuolar membrane H$^+$.ATPase and mitochondrial F$_1$-ATPase. A, purified vacuolar membrane H$^+$.ATPase (5 $\mu$g of protein) and submitochondrial particles (10 $\mu$g of protein) were incubated in standard reaction mixture without substrate but with antiserum for 12 h at 4 $^\circ$C before starting the reaction. B, assays were carried out under standard conditions. The activities of purified vacuolar membrane H$^+$.ATPase (1.5 $\mu$g of protein) and mitochondrial F$_1$-ATPase (2.2 $\mu$g of protein) were measured in the presence of F$_1$-ATPase inhibitor protein at the indicated concentration. A, purified vacuolar membrane H$^+$.ATPase; B, F$_1$-ATPase of submitochondrial particles.

**Fig. 4.** Effects of DCCD (A) and EDAC (B) on purified vacuolar membrane H$^+$.ATPase and H$^+$.ATPases in vacuolar membranes, plasma membranes, and submitochondrial particles. Enzyme samples were incubated with DCCD or EDAC at the concentrations indicated for 25 min at 30 $^\circ$C before starting the reaction. Assays were carried out as described under "Materials and Methods." ——, purified vacuolar membrane H$^+$.ATPase; - - - , mitochondrial F$_1$-ATPase; ——, plasma membranes; and ——, submitochondrial particles.

The activity of purified vacuolar membrane H$^+$.ATPase is more sensitive to the hydrophilic derivative of DCCD, EDAC, than the plasma membrane H$^+$.ATPase or mitochondrial F$_1$-ATPase (Fig. 4B). EDAC at 0.1 mM inhibited the vacuolar membrane H$^+$.ATPase, but reduced the activities of the plasma membrane and mitochondrial enzymes less than 10%. Therefore, EDAC is a useful inhibitor of vacuolar membrane H$^+$.ATPase when used at a proper concentration.

The effects of several other inhibitors of H$^+$.translocating ATPases were examined and the results are summarized in Table III. The activity of purified vacuolar membrane H$^+$.ATPase was strongly inhibited by NBD-Cl and tributyltin, which are known to interact specifically with the $\beta$-subunit of F$_1$.ATPase. The activity was also inhibited by DES and quercetin, but not by sodium azide or oligomycin.

Of the specific inhibitors of plasma membrane H$^+$.ATPase, sodium vanadate and miconazole, which are known to inhibit the dephosphorylation and phosphorylation of plasma membrane H$^+$.ATPase from Schizosaccharomyces pombe (21), did not inhibit purified vacuolar membrane H$^+$.ATPase noticeably (Table III). This suggests that vacuolar membrane H$^+$.ATPase is different from the plasma membrane H$^+$.ATPase. Interestingly, however, both ATPases were inhibited similarly by DES and quercetin.

The activity of purified vacuolar membrane H$^+$.ATPase was stimulated 15% by 50 mM KCl and inhibited 43% by 50 mM KSCN and 72% by 50 mM KNO$_3$. The sodium salts of these anions had similar effects.

The activity of purified vacuolar membrane H$^+$.ATPase was insensitive to ammonium molybdate (0.1 mM), which is
TABLE III

<table>
<thead>
<tr>
<th>Inhibitor (mm)</th>
<th>Purified vacuolar membrane H+-ATPase</th>
<th>Vacuolar membrane H+-ATPase</th>
<th>Plasma membrane H+-ATPase</th>
<th>Mitochondrial H+-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DCCD (0.001)</td>
<td>38</td>
<td>63</td>
<td>86</td>
<td>12</td>
</tr>
<tr>
<td>EDAC (0.1)</td>
<td>77</td>
<td>23</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>NBD-C (0.1)</td>
<td>23</td>
<td>27</td>
<td>79</td>
<td>6</td>
</tr>
<tr>
<td>Tributyltin (0.1)</td>
<td>14</td>
<td>45</td>
<td>33</td>
<td>15</td>
</tr>
<tr>
<td>Sodium azide (2.0)</td>
<td>95</td>
<td>110</td>
<td>105</td>
<td>4</td>
</tr>
<tr>
<td>Oligomycin (0.047)</td>
<td>96</td>
<td>74</td>
<td>74</td>
<td>10</td>
</tr>
<tr>
<td>DES (0.1)</td>
<td>30</td>
<td>48</td>
<td>16</td>
<td>95</td>
</tr>
<tr>
<td>Quercetin (0.1)</td>
<td>37</td>
<td>67</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Sodium vanadate (0.1)</td>
<td>95</td>
<td>96</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>Miconazole (0.2)</td>
<td>106</td>
<td>109</td>
<td>23</td>
<td>46</td>
</tr>
</tbody>
</table>

Relative activity%|  

a Assays were carried out under standard conditions with the indicated inhibitors. In assays with purified vacuolar membrane H+-ATPase, sonicated soybean phospholipids (0.1 mg/ml) were added to the reaction mixture.

b Submitochondrial particles were used as an enzyme source.

Fig. 5. SDS-polyacrylamide gel electrophoresis of purified vacuolar membrane H+-ATPase. The gel contained 15% polyacrylamide and electrophoresis was carried out as described under "Materials and Methods." Lane 1 shows the positions of molecular weight markers of 180,000, 140,000, 100,000, 42,000, and 39,000 of RNA polymerase B from a thermophilic bacterium. Lane 2, vacuolar membranes. Lane 3, EDTA-washed membranes. Lane 4, purified vacuolar membrane H+-ATPase. Arrowheads indicate the positions of polypeptide a (M, = 89,000), b (M, = 64,000), and c (M, = 19,500) as constituent subunits of the enzyme. Lane 5, mitochondrial F1-ATPase.

an inhibitor of acid phosphatase associated with vacuoles of Beta vulgaris L. (22).

Fig. 6. Fluorogram of SDS-polyacrylamide gel showing [14C]DCCD binding polypeptides (indicated by arrowheads) of purified vacuolar membrane H+-ATPase (polypeptide c of M, = 19,500; Lane 1), mitochondrial F1-ATPase (a-subunit of M, = 52,000 and DCCD-binding protein of M, = 8,000; Lane 2), and plasma membrane ATPase (M, = 105,000; Lane 3). Samples were subjected to gel electrophoresis and bands were located by fluorography as described under "Materials and Methods."
DISCUSSION

There were two initial problems in solubilization of the $H^+$-translocating, $Mg^{2+}$-ATPase from vacuolar membranes. One was how to purify vacuolar membranes on a large scale free from contaminating mitochondria and the other was how to remove large amounts of nonspecific alkaline and acid phosphatases, which were soluble contaminating enzymes that were unavoidably associated with the vacuolar membrane vesicle fraction, since they are marker enzymes of intact vacuoles (1). Taking advantage of the differences in sensitivity to sodium azide of the vacuolar membrane and submitochondrial ATPases (1) and to ammonium molybdate of the acid phosphatase and $H^+$-ATPase, we established a method for purifying vacuolar membrane vesicles and then washing them with 1 mM EDTA as described under “Materials and Methods.”

Of the various detergents tested for solubilization, ZW3-14 was found to be only one of practical use. This reagent is also useful for solubilizing several $H^+$-translocating, $Mg^{2+}$-ATPases from plasma membranes of S. cerevisiae (9) and vacuolar membranes of Saccharomyces carlsbergensis (24), plasma membranes of Avena sativa roots (25), and microsomal membranes of Zea mays roots (26). We found that both the concentration of the detergent ZW3-14 and its weight ratio to protein during solubilization and separation by glycerol density gradient centrifugation were critical for recovery of enzyme activity. The procedure described under “Materials and Methods” was one of our best for routine use.

Judging from its differences in sensitivities to various specific inhibitors, vacuolar membrane $H^+$-ATPase differed enzymologically from the $H^+$-ATPases of plasma membranes and mitochondria of S. cerevisiae (Fig. 4 and Table III). In particular, the vacuolar membrane $H^+$-ATPase was distinguishable from mitochondrial $F_1$-ATPase by its insensitivities to antiserum against mitochondrial $F_1$-ATPase and the mitochondrial $F_1$-ATPase inhibitor protein (Fig. 3).

From the results of polypeptide analysis of the partially purified enzyme (Figs. 5 and 6), we propose that the vacuolar membrane $H^+$-ATPase is composed of two large polypeptides, $a$ and $b$ ($M_r = 89,000$ and $64,000$), and one small, DCCD-binding polypeptide $c$ ($M_r = 19,500$). This finding suggests that the subunit composition of the enzyme is entirely different from that of $H^+$-ATPase from the plasma membrane of S. cerevisiae (9, 10). We are investigating the stoichiometry of polypeptides $a$, $b$, $c$, and others, if any, in the purified enzyme preparation to compare the unique properties of the vacuolar membrane $H^+$-ATPase of S. cerevisiae with those of other vacuolar membranes (24, 27, 28) and plasma membrane $H^+$-ATPases from other sources (29).

Acknowledgments—We are indebted to Dr. K. Tagawa for kindly providing us with antiserum against mitochondrial $F_1$-ATPase and purified mitochondrial $F_1$-ATPase inhibitor protein.

REFERENCES