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Purification and Properties of the Plasma Membrane H^+-Translocating Adenosine Triphosphatase of *Phaseolus mungo* L. Roots[1]

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ABSTRACT

The plasma membrane ATPase of mung bean (*Phaseolus mungo* L.) roots has been solubilized with a two-step procedure using the anionic detergent, deoxycholate (DOC) and the zwitterionic detergent, zwittergent 3-14 as follows: (a) loosely bound membrane proteins are removed by treatment with 0.1% DOC; (b) The ATPase is solubilized with 0.1% zwittergent in the presence of 1% DOC; (c) the solubilized material is further purified by centrifugation through a glycerol gradient (45–70%). Typically, about 10% of the ATPase activity is recovered, and the specific activity increases about 11-fold. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis shows that the peak fraction from the glycerol gradient contains three major polypeptides of M_r = 105,000, 67,000, and 57,000 daltons. The properties of the purified ATPase are essentially the same as those of membrane-bound ATPase, with respect to pH optimum, substrate specificity, inhibitor sensitivity, and ion stimulation.

The existence of an electrogenic H^+-translocating ATPase in plant plasma membranes has been postulated on the basis of electrophysiological and biochemical evidence (29, 31, 32, 34, 35). In support of this notion, studies with isolated membrane vesicles have shown by the ATP-dependent formation of both a membrane potential and a pH gradient (35). If the ATPase could be solubilized and purified, it might be possible to reconstitute and analyze further its structure and transport properties. Despite rapid advances in the purification and characterization of transport proteins from biological membranes (17, 36), however, there are few examples of purified, well characterized transport proteins from plant plasma membranes (2, 10–12, 18, 33, 38) and reconstitution of H^+-ATPase (27, 30, 38).

Recently there have been several reports of the purification of plant plasma membrane-bound ATPase using a variety of detergents for solubilization: deoxycholate, CHAPSO[2] or octylglucoside in the presence of glycerol (27), cholate followed by sucrose density gradient centrifugation (10), or neutral zwittergent followed by precipitation with $(NH_4)_2SO_4$ (38). In the present study, H^+-translocating ATPase was solubilized in two steps using DOC and zwittergent 3-14 in the presence of glycerol, and purified by glycerol-gradient centrifugation. The characteristics of the purified enzyme were also investigated.

MATERIALS AND METHODS

Plant Material. Mung bean (*Phaseolus mungo* L.) seeds were germinated on thin absorbent cotton on a 0.75% agar plate in a enameled tray. After cultivation for 75 h in the dark at 32°C, roots were excised and chilled in aerated cold distilled H_2O.

Isolation of Plasma Membrane Fraction. Plasma membrane-enriched fractions from a 10,000 to 80,000*g* pellet were isolated as described previously (20) with minor modifications. Briefly, to increase the yield, microsomes were layered on a 20/30/40% (w/w) discontinuous sucrose gradient and centrifuged at 80,000*g* for 2 h. Membrane fractions were collected from the 30/40% interface, and diluted with a medium containing 1 mM DTT, 1 mM EDTA, and 2.5 mM Hepes-Tris (pH 7.3). The membranes were centrifuged at 100,000*g* for 40 min. The resulting pellets were suspended in medium containing 0.25 M mannitol, 1 mM DTT, and 2.5 mM Hepes-Tris (pH 6.5) to a concentration of 4 to 6 mg protein/ml, and stored at −80°C for up to 1 week.

Detergent Treatment of Plasma Membrane Fractions. The frozen plasma membrane fraction was thawed and diluted with suspension buffer containing 0.2 M KCl, 2 mM EDTA, 25 mM Tris-HCl (pH 7.5) to a protein concentration of 2 mg/ml. A two-step solubilization procedure using DOC (Nakarai Chemical, Tokyo) and zwittergent 3-14 (Calbiochem) was employed. In the first step, DOC was added drop-by-drop from a 10% stock solution to give a final concentration of 0.1% DOC; the plasma membrane suspension was maintained at 0°C with constant stirring during this step. The mixture was then centrifuged at 100,000*g* for 1 h. Most of the ATPase remained in the pellet, which was suspended at a protein concentration of 2 mg/ml in a solution containing 2 mM EDTA, 0.3 M KCl, 45% glycerol (v/v), and 25 mM Tris-HCl (pH 7.5). In the second step, DOC and zwittergent were added to the suspension to a final concentration of 1% and 0.1%, respectively, again with constant stirring at 0°C. This mixture was centrifuged at 100,000*g* for 30 min, after which the supernatant was diluted by adding an equal volume of solution containing 1 mM EGTA-Tris (pH 7.5) and centrifuged at 105,000*g* for 4 h. The final pellet was suspended in 0.4 ml of 1 mM EGTA-Tris (pH 7.5), and then layered on the top of a 5 ml linear gradient of glycerol (45–70%) containing 0.3% DOC, 10 mM Tris-HCl (pH 7.5), and 2 mM EGTA. Gradients were centrifuged at 150,000*g* for 15 h. One-half-ml fractions were taken from the bottom using a Perista pump (Atto Co., Tokyo) and assayed for protein and ATPase activity. The fractions containing high ATPase activity were collected together and

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[2] Abbreviations: CHAPSO, *N,N'*-[(octanoylimino)bis(trimethylene)]-bis(D-gluconamide); DCCD, *N,N'*-dicyclohexylcarbodiimide; DES, diethylstilbestrol; DOC, deoxycholate.

Table I. *ATPase Purification and Yield*

ATPase activity was determined in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and in the absence and presence of 0.03% asolectin. Yields were calculated on the basis of activity in the presence of asolectin.

Fraction	ATPase Activity				Protein	
	Asolectin		Yield		Amount	Yield
	−	+	Unit	%		
	μmol Pi/mg protein·min		*μmol Pi/min*		*(mg)*	*(%)*
Plasma membrane	0.41	0.54 (1)	3.53	100	6.51	100
0.1% DOC pellet	0.76	1.38 (2.5)	3.59	101.7	2.61	40.1
1% DOC supernatant	1.34	1.78 (3.3)	0.63	17.8	0.35	5.4
Glycerol gradient peak fractions	1.55	2.32 (4.3)	0.17	4.8	0.07	1.1
Plasma membrane	0.31	0.39 (1)	1.82	100	4.67	100
0.1% DOC pellet	0.66	0.91 (2.3)	1.89	103.8	2.07	44.3
0.1% Zwittergent 3-14 supernatant	1.04	1.10 (2.8)	0.39	21.4	0.36	7.6
Glycerol gradient peak fractions	1.13	1.26 (3.2)	0.14	7.7	0.15	3.3
Plasma membrane	0.52	0.58 (1)	2.77	100	4.14	100
0.1% DOC pellet	1.02	1.43 (2.4)	2.93	105.8	1.70	41.1
0.1% Zwittergent 3-14 + 1% DOC supernatant	2.61	3.61 (6.2)	0.87	31.4	0.60	14.5
Glycerol gradient peak fractions	2.73	6.23 (10.7)	0.27	9.7	0.12	2.9

diluted with an equal volume of 1 mM EGTA-Tris (pH 7.5) and centrifuged at 170,000*g* for 4 h. The resulting pellet was suspended in either 25 mM Tris-HCl buffer (pH 7.5) containing 0.3 M KCl, 2 mM EDTA, and 45% glycerol or 1 mM EGTA-Tris (pH 7.5) in the presence of 0.05% asolectin (Associated Concentrates Inc., New York). This material will be termed glycerol gradient purified ATPase.

ATPase Assay. ATPase activity was routinely measured at 38°C for 30 min with 1 to 5 μg protein per assay (20). The reaction was carried out in a volume of 0.5 ml containing 3 mM Tris-ATP (Boehringer), 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5) in the presence of 0.03% asolectin. Variations in assay of pH or ion content are indicated in the text or legends. Phosphate was determined by the method of described previously (19).

SDS-PAGE. SDS-PAGE was carried out according to Laemmli (22) using 1.0 mm thick, 12.5% acrylamide running gels and 4.5% acrylamide stacking gels. Proteins to be loaded onto the gel were suspended in a solution containing 2% SDS, 1% β-mercaptoethanol, 0.12 M Tris (pH 6.8), and heated in a boiling water bath for 2 to 3 min. The running buffer was 0.2 M glycine, 0.25 M Tris, and 0.1% SDS. The gels were stained and destained by the method of Fairbanks *et al.* (13).

Protein Assay. Protein was determined by the method of Bradford (6) with BSA as a standard.

RESULTS

Detergent Treatment of the Plasma Membrane Fraction. Plasma membrane-bound ATPase was solubilized by a two-step procedure using DOC and zwittergent. Table I is a summary of the recoveries of enzyme activity and protein during purification. In the first step, 0.1% DOC was employed to remove loosely bound membrane proteins. Typically, about 60 to 55% of the protein was removed but close to 100% of the ATPase activity remained in the pellet. This step had the effect of increasing the selectivity of subsequent detergent solubilization. In the next step, solubilization of the ATPase from extracted membranes was attempted with DOC and/or zwittergent in the presence of glycerol. When zwittergent (0.1%) or DOC (1%) was used singly, the final supernatant contained 18 to 21% of the ATPase, with a 2.8- to 3.3-fold increase in specific activity. By contrast, when zwittergent and DOC were used together, the final supernatant contained 31% of the starting ATPase and had a nearly 6.2-fold increase in specific activity. Thus, unless otherwise stated, the ATPase in 0.1% DOC extracted membranes was solubilized with 0.1% zwittergent in the presence of 1% DOC.

Purification of Glycerol Gradient Centrifugation. The solubilized ATPase could be partially purified by centrifugation through a linear gradient of glycerol (45–70%) in the presence of 0.3% DOC. Figure 2 illustrates the distribution of protein and ATPase activity in such a gradient which was centrifgued for 15 h at 150,000*g*. The final purification procedure is diagramed (Fig. 1) and the results of a representative purification are summarized in Table I. In this experiment, the specific activity of the ATPase increased from 0.6 μmol Pi/mg protein·min in the starting plasma membranes to 1.4 μmol Pi/mg protein·min after treatment with 0.1% DOC, 3.6 μmol Pi/mg protein·min following solubilization with 0.1% zwittergent in the presence of 1% DOC, and finally 6.2 μmol Pi/mg protein·min in the pooled fractions from the glycerol gradient. The final recovery was about 10% although there was some loss of enzyme units at each step. Under these conditions, about 21% of total ATPase still remained in the glycerol gradient pellet without inactivation of the enzyme. The properties of the ATPase in the gradient pellet were found to be similar to the glycerol gradient purified enzyme with respect to vanadate inhibition and optimum pH (data not shown).

The protein composition of the peak fraction from the glycerol gradient was examined by SDS-PAGE (Fig. 3). There were three major polypeptides of M_r = 105,000, 67,000, and 57,000 and two minor polypeptides of M_r = 36,000 and more than 120,000 D.

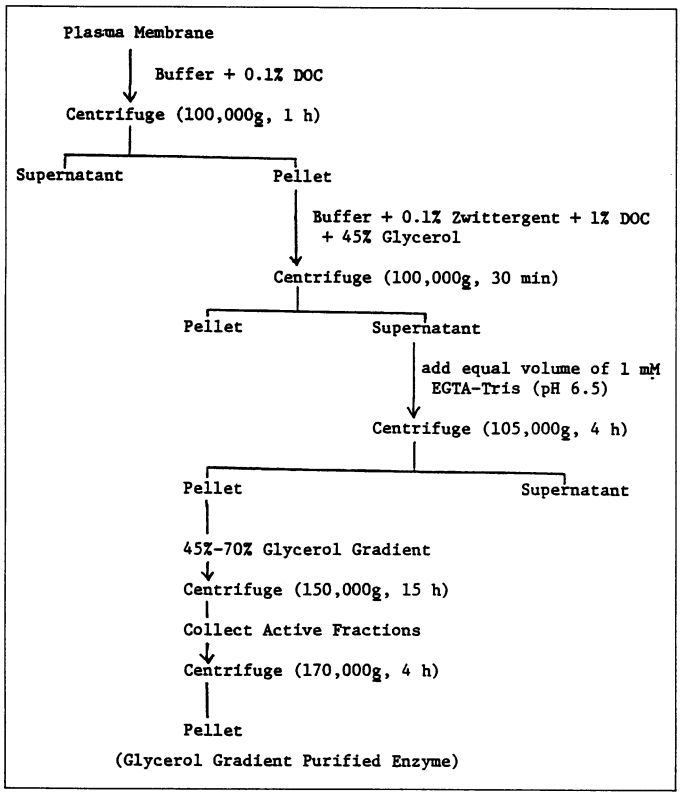

FIG. 1. Flow chart for the purification of plasma membrane ATPase. The composition of the buffer at each step is given under "Materials and Methods."

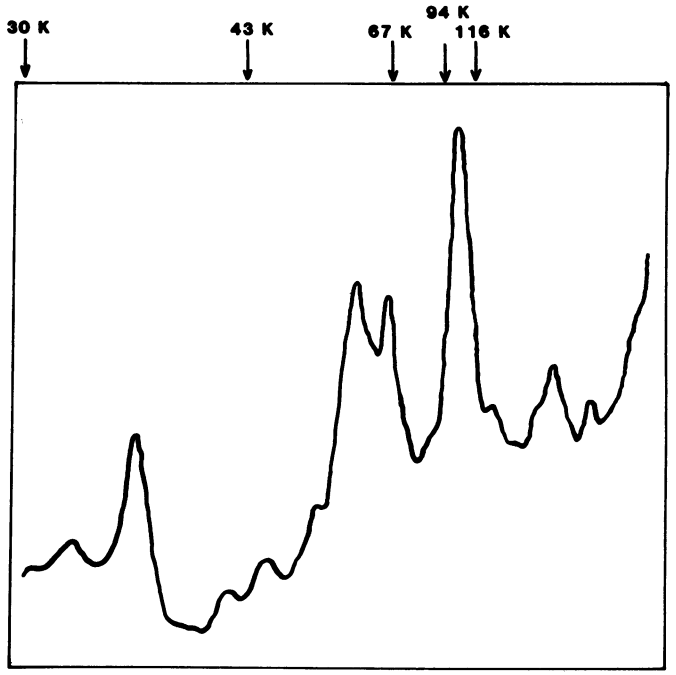

FIG. 3. SDS-polyacrylamide gel profiles of glycerol gradient purified ATPase. Gel was stained with Coomassie blue and scaned at 560 nm with a Shimadzu Dual-Wavelength TLC scanner (model CS-920). Molecular weights were estimated from a plot of log mol wt *versus* relative mobility using gels of the following protein standards of Pharmacia: 14.4 K, α-lactalbumin; 20.1 K, trypsin inhibitor; 30.0 K, carbonic anhydrase; 43 K, ovalbumin; 67 K, albumin; 94 K, phosphohydrolase; 11.6 K, β-galactosidase.

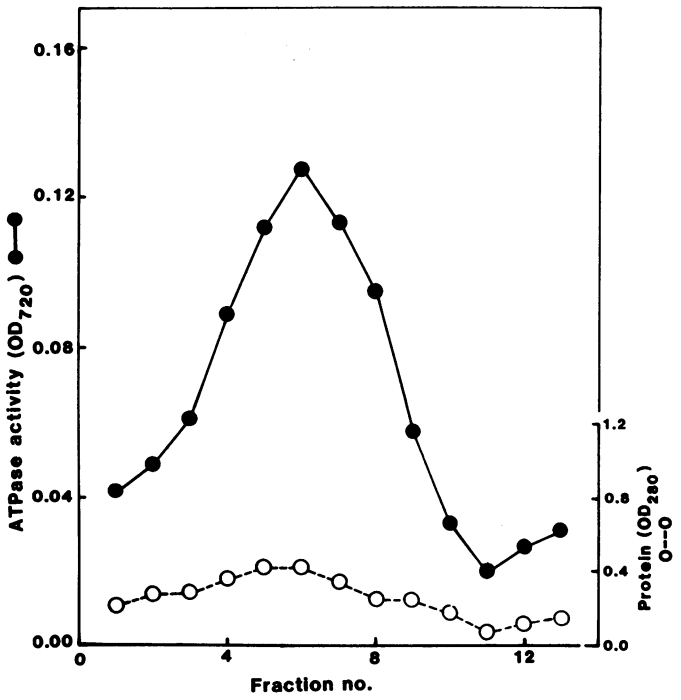

FIG. 2. Distribution of zwittergent and DOC-solubilized ATPase (●——●) and protein (○- - -○), on a linear 45 to 70% glycerol gradient after centrifugation for 15 h at 150,000*g*. Vanadate-sensitive ATPase activity was measured in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), 0.03% asolectin, and in the presence or absence of 10 μM vanadate.

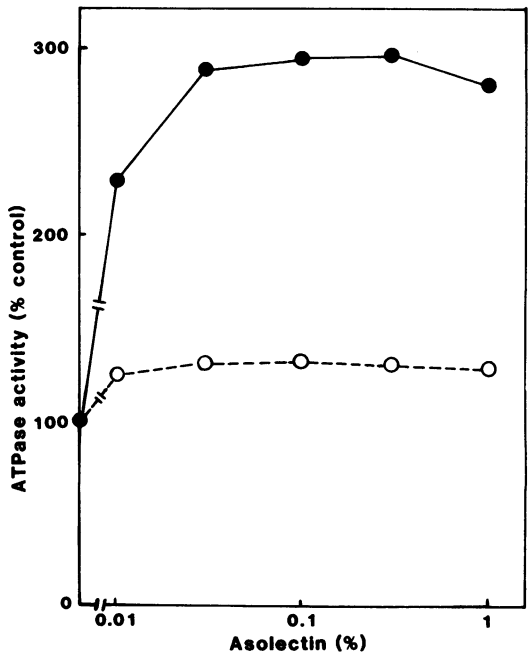

FIG. 4. Stimulation of the glycerol gradient purified ATPase from 1% DOC-solubilized fraction (●——●) and 0.1% Zwittergent -solubilized fraction (○- - -○) by phospholipid. ATPase activity was assayed in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), and various concentrations of asolectin as indicated. Control activities in the absence of added asolectin were 0.66 (DOC) and 1.50 μmol Pi/mg protein·min (Zwittergent).

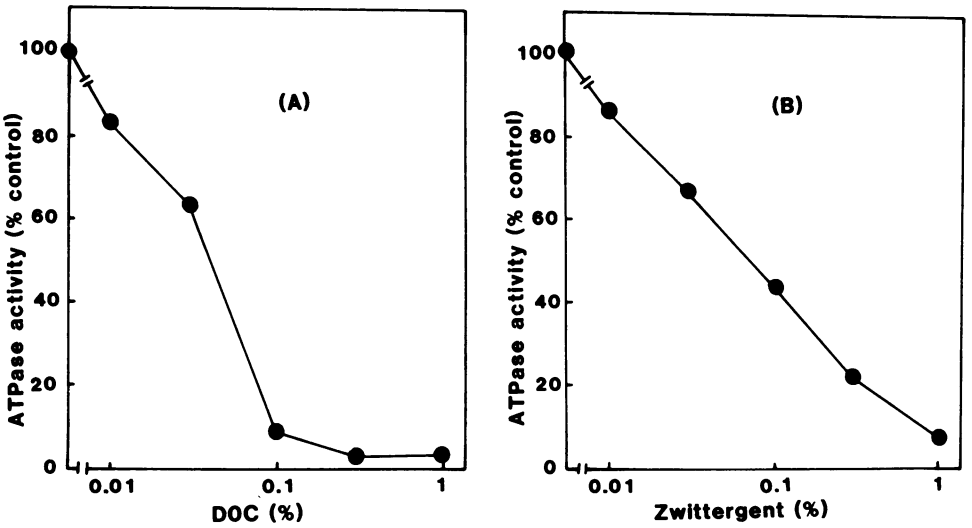

FIG. 5. Effects of DOC and Zwittergent 3-14 on glycerol gradient purified ATPase from the fraction solubilized with 1% DOC (A) and 0.1% Zwittergent (B). ATPase activity was assayed in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 0.03% asolectin, and various concentrations of DOC and Zwittergent 3-14. Control activities without DOC and Zwittergent were 2.06 and 1.72 μmol Pi/mg protein·min, respectively.

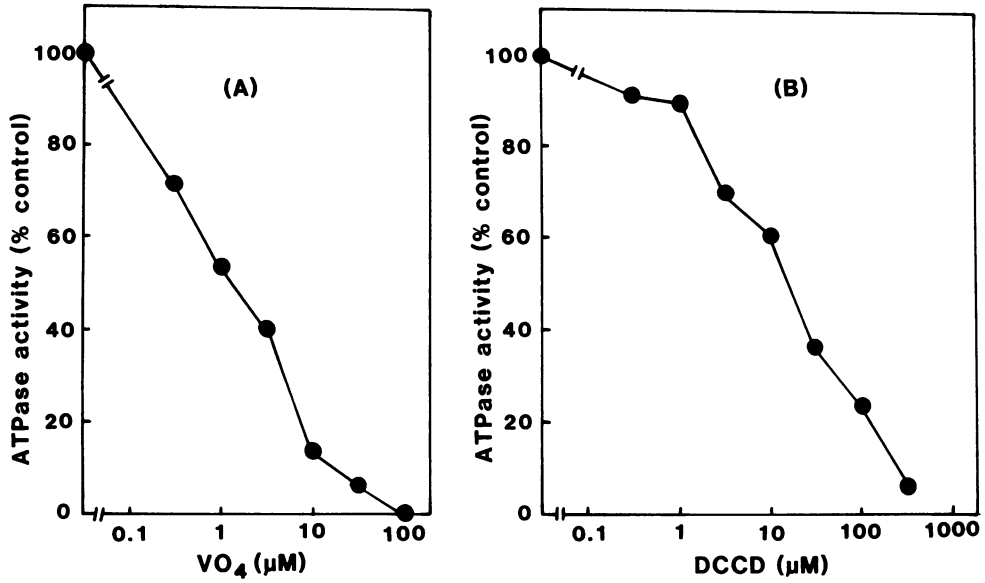

FIG. 6. Effects of VO_4 (A) and DCCD (B) on glycerol gradient purified ATPase. ATPase activity was assayed in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 0.03% asolectin, and various concentrations of VO_4 and DCCD. Control activities without VO_4 and DCCD were 2.11 and 3.14 μmol Pi/mg protein·min, respectively.

Effects of Phospholipids and Detergents on ATPase Activity. Following purification, ATPase activity increased upon the addition of phospholipid to the assay mixture. In the case of enzyme solubilized with 0.1% zwittergent in the presence of 1% DOC, there was more than 2-fold stimulation by asolectin (Table I). Optimum stimulation was seen at an asolectin concentration of 0.3 to 0.3% (Fig. 4). The same concentrations of asolectin produced a 50 to 290% stimulation (Table I; Fig. 4) of ATPase solubilized with 0.1% DOC alone, and a 12 to 31% stimulation of enzyme with 0.1% zwittergent alone (Table I; Fig. 4).

Purified ATPase was sensitive to DOC and zwittergent, with half-maximal inhibition at 0.03 and 0.05%, respectively (Fig. 5).

Effects of Various Inhibitors on Purified ATPase. It is well known that the H^+-translocating ATPase of fungal plasma membranes is inhibited by vanadate (4). Vanadate (Wako Chemical Co., Osaka or Fisher) completely inhibited glycerol gradient purified ATPase from mung bean (Fig. 6A), with half maximal inhibition at 1.0 μM. The purified ATPase also retained its sensitivity to DCCD (Aldrich), an inhibitor known to react with the proton channels of chloroplast and bacterial ATPase (14, 26). The concentration required for half maximal inhibition was about 10 μM (Fig. 6B).

The effects of various phosphohydrolase inhibitors on the purified ATPase activity were examined (Table II). The activity was insensitive to oligomycin (Sigma) and azide, the mitochondrial ATPase inhibitors and ouabain (Aldrich), a specific inhibitor of Na^+-K^+-ATPase. Molybdate, an inhibitor of nonspecific phosphatase in plant cells, did not affect the activity. Nitrate, an inhibitor of tonoplast ATPase in plant cells, slightly affected the activity while DCCD, DES (Sigma) vanadate, tyrocidine (United States Biochemical Corp., Ohio), and gramicidin S (Sigma), inhibitors of plasma membrane ATPase (20), strongly inhibited the enzyme.

Other Characteristics of the Purified Enzyme. The enzymic properties of the purified ATPase were examined so that they could be compared with the previously reported properties of the membrane-bound enzyme (20). The pH optimum for the ATPase was 6.5 (Fig. 7). Table III shows that the purified ATPase was activated by divalent cations in the presence of 50 mM KCl in the following order: $Mg^{2+}>Mn^{2+}>Co^{2+}>Cu^{2+}$. Maximal effect of Mg^{2+} on the activity of the purified ATPase occurred at a concentration of 1.5 to 3.0 mM (data not shown). The lower part of Table III shows the effect of monovalent cations. Stimulation exhibited the following order: $K^+>Rb^+>NH_4^+>Na^+>Li^+>$choline$>Cs^+$. The purified ATPase activity was decreased with increasing concentrations of CsCl (>30 mM, data not shown). Maximal stimulation by KCl (40 mM) was 80% (Fig. 8). Table IV illustrates the substrate specificity of the purified ATPase. The

Table II. *Effects of Inhibitors on Purified ATPase*

ATPase activity was measured in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), 0.03% asolectin, and various concentrations of inhibitor as indicated. Numbers in parentheses indicate % of control.

Inhibitor	ATPase Activity
	(μmol Pi/mg protein·min)
None	4.19 (100)
Oligomycin (10 μg/ml)	3.98 (95.0)
NaN_3 (1 mM)	3.82 (91.2)
Ammonium molybdate (100 μM)	3.99 (95.2)
Ouabain (50 μM)	3.85 (91.9)
KNO_3 (100 mM)	3.37 (80.5)
DCCD (100 μM)	1.40 (33.4)
DES (100 μM)	1.55 (36.9)
Vanadate (50 μM)	0.39 (9.3)
Tyrocidine (50 μM)	2.25 (53.7)
Gramicidin S (100 μM)	1.59 (38.0)

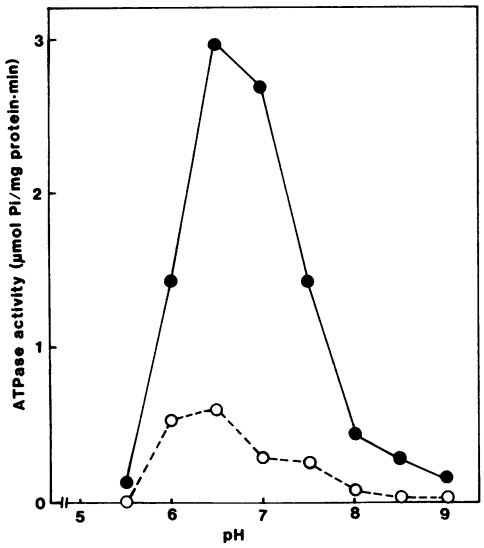

FIG. 7. pH dependence of glycerol gradient purified ATPase. ATPase activity was assayed in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 50 mM KCl, 0.03% asolectin, and in the presence (O- - -O) or absence (●——●) of 10 μM VO_4. The assay pH was adjusted by varying the ratio of Tris/Mes.

enzyme actively hydrolyzed ATP, and another nucleotides, *p*-nitrophenylphosphate and β-glycerophosphate were hydrolyzed to a lesser degree. These characteristics of the purified enzyme were essentially the same as those of membrane-bound enzyme (20).

DISCUSSION

A previous paper (20) from this laboratory demonstrated the existence of an H^+-translocating ATPase in plasma membrane vesicles of *Phaseolus mungo* L. roots. As valuable as the vesicle studies have been, it is also important to purify the plasma membrane ATPase and determine its molecular properties.

A two-step solubilization procedure was originally used by Kyte (21) for the Na^+-K^+-ATPase of dog kidney. Similar procedures were followed by Bowman *et al.* (3) with *Neurospora* ATPase and Briskin and Poole (8) with red beet ATPase. The principle of the procedure is to use a low concentration of DOC to remove loosely bound membrane proteins, leaving a membrane fraction enriched in ATPase. Then, DOC-extracted membrane pellets are disrupted with a higher concentration of DOC or another suitable detergent, extracting the ATPase in a form that can be separated from other membrane components.

Table III. *Effects of Divalent and Monovalent Cations on Glycerol Gradient Purified ATPase*

Glycerol gradient purified ATPase activity was assayed in the presence of 3 mM Tris-ATP, 3 mM divalent cations, 50 mM monovalent cations, and 30 mM Mes-Tris (pH 6.5).

Cation	ATPase Activity
	(μmol Pi/mg protein·min)
KCl	
+ $MgSO_4$	4.66 (100)
+ $MnSO_4$	2.64 (56.5)
+ $CoCl_2$	2.30 (49.3)
+ $ZnSO_4$	0 (0)
+ $CaCl_2$	0 (0)
+ $CuSO_4$	1.96 (42.0)
$MgCl_2$	
+ KCl	5.64 (100)
+ RbCl	5.54 (98.2)
+ CsCl	1.08 (19.2)
+ NaCl	5.00 (88.6)
+ LiCl	3.60 (63.5)
+ Choline-Cl	2.82 (50.3)
+ NH_4Cl	5.34 (94.6)

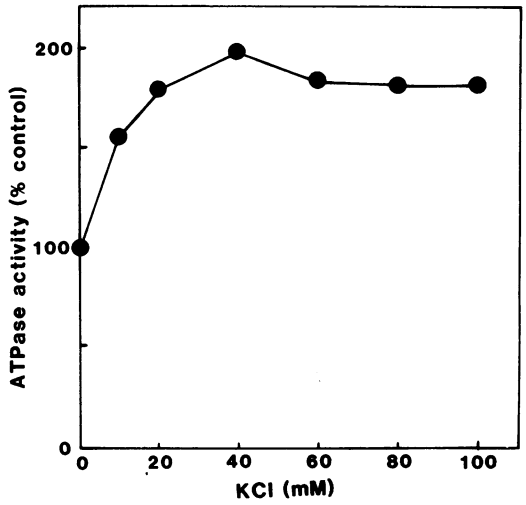

FIG. 8. Effect of KCl on glycerol gradient purified ATPase. ATPase activity was assayed in the presence of 3 mM $MgSO_4$, 3 mM Tris-ATP, 30 mM Mes-Tris (pH 6.5), 0.03% asolectin, and various concentrations of KCl as indicated. The control activity without KCl was 1.52 μmol Pi/mg protein·min.

In the present work, 0.1% DOC was used to remove loosely bound membrane proteins. This procedure proved very effective, because 40 to 60% of membrane proteins were removed but ATPase was not (Table I), resulting in a 2-fold increase of specific activity. The second step was treatment with a mixture of 1% DOC and 0.1% zwittergent in the presence of 45% glycerol. The fraction solubilized had higher specific activity, and could be obtained in better yield than with either DOC or zwittergent alone (Table I). Zwittergent has also been used in the purification of yeast membrane ATPase (23) and in the partial purification of the plasma membrane ATPase from oat roots (38). Because DOC and zwittergent can both inhibit ATPase activity (Fig. 5),

Table IV. *Substrate Specificity of Glycerol Gradient Purified ATPase*

Enzymic activity was assayed in the presence of 3 mM $MgSO_4$, 50 mM KCl, 30 mM Mes-Tris (pH 6.5), 0.03% asolectin in the presence of 3 mM nucleotide.

Substrate	Enzymic Activity
	(μmol Pi/mg protein·min)
AMP	0.06 (1.8)
ADP	0.34 (8.8)
ATP	3.92 (100.0)
GTP	1.22 (31.0)
UTP	1.18 (30.1)
CTP	0.72 (18.6)
β-Glycerol-P	0.18 (4.4)
p-Nitrophenyl-p	0.18 (4.4)

contact of the purified enzyme with detergent was limited to 30 min in order to obtain maximal activity (see "Materials and Methods"). Glycerol, which has been used to protect certain other membrane ATPases from inactivation by detergent (3, 8, 16, 37), was also required. The specific activity remained fairly constant for 2 to 3 h if the preparation was maintained at ice temperature in the presence of glycerol, but virtually all of the purified ATPase was inactivated in the absence of glycerol (data not shown). In subsequent experiments, the glycerol concentration was fixed at 45% and protein and DOC/or zwittergent were varied in order to identify optimum concentrations. The best results were obtained with 1% DOC plus 0.1% zwittergent, with a membrane protein concentration of 2.0 mg/ml, and with a detergent to protein ratio of 1.0 (mg/mg) (data not shown). Under these conditions, about 31% of ATPase appeared in the supernatant (Table I). If increase in the ATPase is due to the amount of protein solubilized, specific activity in glycerol peak should be increased by about 20 μmol Pi/mg protein·min, but the value was 6.2 in the preparation of this work (Table I). This shows some loss of enzyme unit during purification, especially during glycerol gradient step or storage at ice temperature (8). During ATPase assay, addition of phospholipid is absolutely required for maximal activity of DOC-solubilized ATPase, but less important for zwittergent-solubilized enzyme. Optimum stimulation was seen at an asolectin concentration of 0.03 to 0.1% (Fig. 4). Asolectin used in the present work was prepared from soybean lecithin and contains 95 to 98% phospholipids. Bennett and Spanswick (1) showed that the activation by added phospholipid is relatively nonspecific and results from hydrophobic interaction between the H^+-ATPase and lipid environment.

An identifying characteristic of the plasma membrane-bound ATPase from mung bean roots is high sensitivity to vanadate (Fig. 6A), and in this respect it resembles the *Neurospora* H^+-ATPase (5), the Na^+-K^+-ATPase of animal cells (9), and other plant plasma membrane ATPase (15). The purified mung bean ATPase was inhibited by vanadate with $[I]_{0.5}$ value of 1.0 μM. The pH optimum was 6.5 (Fig. 7). Ion specificity (Table III) and substrate specificities (Table IV) were similar to those of the membrane-bound ATPase (20).

Serrano (30) obtained ATPase preparation which gave a single 100,000 D peak on SDS gel, while analysis of glycerol gradient purified ATPase by SDS-PAGE in this paper revealed three major polypeptides of 105,000, 67,000, and 57,000 D. Two minor polypeptides of 37,000 and more than 120,000 were sometimes observed (Fig. 3). The polypeptides at 57,000 and 67,000 D may correspond to the prominent 60,000 and 70,000 D polypeptides observed by Mandala and Taiz (24) in partially purified tonoplast ATPase from corn coleoptiles and Manolson *et al.* (25) in the tonoplast one from red beet. Contamination of mitochondria F_1 ATPase and γ subunit of Na^+-K^+-ATPase may be ruled out by the complete lack of inhibition of the enzyme by inhibitors (Table II). Thus, one possible contaminant may be the tonoplast subunits. In addition, nitrate slightly inhibited the purified enzyme (Table II). The 105,000 D polypeptide is reminiscent of the catalytic subunit of the fungal plasma membrane H^+-ATPase and the Na^+-K^+-ATPase of animal cells, and is also similar in mol wt to the polypeptide that is phosphorylated from γ-^{32}P-ATP in plant plasma membranes (7, 39). Whether any of the other polypeptides represent true ATPase subunits remains to be determined. Recently, more detailed molecular analysis of the similarity between H^+-ATPase of plant plasma membrane, Na^+-K^+-ATPase (28) and gastric H^+-K^+-ATPase including the formation of phosphate intermediate and DCCD-binding site have been shown (40).

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