Plant Physiol. (1986) 81, 1080–1085
0032-0889/86/81/1080/06/$01.00/0

Purification and Properties of the H^+-Translocating ATPase from the Plasma Membrane of Tomato Roots[1]

Received for publication February 18, 1986 and in revised form April 14, 1986

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ABSTRACT

The proton-translocating, plasma membrane ATPase was purified from tomato roots. At the final stage of purification approximately 80% of the protein was found in a single band with an apparent molecular weight of 90 kilodaltons. Cross-linking studies indicated that the ATPase normally exists as a trimer of catalytic subunits. No evidence was found for any additional subunits. The pH optimum for ATP hydrolysis by the purified protein was 6.5. Activity was stimulated by K^+, especially at low pH, and inhibited by vanadate, *N,N'*-dicyclohexylcarbodiimide, and diethylstilbestrol; nitrate was weakly inhibitory. Activity was stimulated by lysolecithin but inhibited by sonicated phospholipids. The inhibition by lipids could be prevented if octylglucoside was added with the lipids; the combination of octylglucoside and lipids actually stimulated activity. The purified protein could be reconstituted into liposomes and catalyzed ATP-dependent, vanadate-sensitive proton translocation.

Plasma membranes of higher plants have been shown to contain an electrogenic H^+-translocating ATPase (17, 22). This ATPase is easily distinguished from other H^+-ATPases found in plants in that it forms a phosphorylated intermediate and is inhibited by VO_4. Analysis of the phosphorylated intermediate has indicated that the enzyme has a catalytic subunit with a mol wt of 100 kD (3, 24). These properties indicate that the plasma membrane ATPase of plants is quite similar in structure and mechanism to both the H^+-ATPases in the plasma membrane of fungi and a variety of cation transport ATPases found in the plasma membrane of animal cells.

To date, most studies of the plant plasma membrane ATPase have been done with the membrane-bound form of the enzyme in partially purified plasma membrane preparations. While such studies are useful, they are nevertheless limited by the fact that the system contains many components other than the ATPase. To approach many questions as to the structure and mechanism of the ATPase unambiguously, it is obviously necessary to obtain the ATPase in pure form. Several reports of solubilization and partial purification of the ATPase have appeared (5, 9, 12, 21, 23). These reports differ from each other both in the degree of purification obtained and in the apparent mol wt of the purified enzyme. The most highly purified preparation reported so far is that obtained by Serrano (21) with oat roots. This preparation had more than 70% of the total protein in a single band at 100 kD, the mol wt of the previously identified catalytic subunit.

Preliminary screening in this laboratory indicated that tomato roots contained large amounts of plasma membrane ATPase activity and thus might serve as an excellent source for the purification of the enzyme. We report here a procedure for obtaining a highly pure and highly active preparation of the plasma membrane ATPase from tomato roots.

MATERIALS AND METHODS

Plant Material. Tomato seeds (*Lycopersicon esculentum* cv Rutgers) were germinated on wire screens over a solution containing 1 mM $CaSO_4$, 0.1 mM KCl, and trace elements as described in Forsberg (10). Roots were harvested after 1 week at 27°C in the dark.

Purification of the ATPase. Approximately 40 g of roots were homogenized in 80 ml of an ice-cold medium containing: 350 mM sucrose, 25 mM MOPS[2]-KOH (pH 7.0), 2.5 mM DTT, 0.1% (w/v) BSA, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM EGTA. The roots were first chopped into fine pieces with a pair of scissors then homogenized for 15 s with a polytron (Brinkman Instruments, Westbury, NY). The homogenate was filtered through six layers of cheese cloth and centrifuged for 10 min at 3,400*g*. The pellet was discarded, and the supernatant centrifuged at 35,000*g* for 30 min. This pellet was resuspended in 25 mM MOPS-KOH (pH 7.0), 2 mM DTT, and 25% (v/v) glycerol (resuspension buffer). These crude membranes could be stored at −70°C without loss of activity. Generally, the membranes from six preparations (about 250 g roots) were pooled before continuing to the next step.

Membranes were diluted with resuspension buffer to a protein concentration of 1 mg/ml and KBr was added to 0.5 M and Triton X-100 to 0.1%. After standing for 20 min on ice, the membranes were pelleted by centrifugation for 30 min at 120,000*g*. This pellet was resuspended in 100 mM KCl, 25 mM MOPS-KOH (pH 7.0), 1 mM DTT, and 30% glycerol to a protein concentration of about 2 mg/ml, then octylglucoside was slowly added to a final concentration of 1% and deoxycholate to 0.2%. After 10 min on ice, the membranes were pelleted and resuspended in 2 ml of resuspension buffer. To this was added 0.4 ml of 100 mg/ml lysolecithin (Sigma, type I) and, after 10 min at room temperature, the mixture was centrifuged for 30 min at 100,000*g* and the supernatant collected. The supernatant was diluted with 1.2 ml H_2O, then 0.8 ml was overlayered on each of four 4 ml 20 to 50% linear glycerol gradients. In addition to glycerol, the gradients contained: 25 mM MOPS-KOH (pH 7.0), 1 mM DTT, 0.2 mg/ml phospholipids (Sigma, type IV), and 0.1% octylglucoside. The gradients were centrifuged for 18 h at 200,000*g* in a Beckman sw 50.1 rotor, then fractionated by upward displacement with 100% glycerol. Approximately 15 fractions were collected with the ATPase activity recovered in

[1] This work supported by National Science Foundation grant DBM-8415035 to R. M. S.

[2] Abbreviations: MOPS, 3-[*N*-morpholino]propanesulfonic acid; BTP, 1,3-bis(tris[hydroxymethyl]amino)propane; DES, diethylstilbestrol; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone.

three or four fractions. The active fractions were pooled and stored at −70°C with no loss of activity for at least 2 months.

ATPase Assays. ATPase activity was measured in 0.5 ml of a medium containing 50 mM Mes-BTP (pH 6.5), 50 mM KCl, 3 mM $MgCl_2$, 2 mM ATP, and 0.05 mg/ml lysolecithin. Assays were started by the addition of 5 μl of the enzyme preparation and allowed to proceed for 10 min at 30°C. Reactions were stopped and Pi determined colorimetrically by the addition of 0.5 ml of a solution based on the method of LeBel et al. (15). This solution contained: 2.4 M acetic acid, 0.4 M sodium acetate, 12 mM $CuSO_4$, 1% (w/v) NH_4MoO_4, 1% (w/v) Na_2SO_3, 0.4% (w/v) *p*-methylaminophenol sulfate, and 1% SDS. After 10 min at room temperature, 0.5 ml of 5% (w/v) sodium citrate was added to prevent any further color development due to nonenzymic hydrolysis of ATP (14).

Reconstitution. Reconstitution of the ATPase into proteoliposomes was done by the freeze-thaw-sonication procedure, as described in Vara and Serrano (23). Lipids (Sigma type II-S) were suspended at 40 mg/ml in 10 mM Mes-BTP (pH 6.5), 50 mM KCl then sonicated to clarity. Equal volumes of lipids and gradient-purified protein were mixed then frozen at −70°C. After 15 min the mixture was thawed and sonicated for 1 min in a bath-type sonicator. The freeze-thaw-sonication procedure was repeated once. Proton pumping was monitored by measuring quinacrine fluorescence quenching (19). Reconstituted proteoliposomes containing approximately 12 μg protein were suspended in a medium containing 10 mM Mes-BTP (pH 6.5), 50 mM KBr, 6 mM $MgCl_2$, and 10 μM quinacrine. Once a stable baseline was obtained, ATP was added to a final concentration of 5 mM.

Cross-Linking and Electrophoresis. Cross-linking was done essentially as described by Craig (7). Purified protein at approximately 0.15 mg/ml was taken directly from the glycerol gradient and incubated at room temperature with glutaraldehyde. After 1 h, a 10-fold excess of $NaBH_4$ was added to reduce the unreacted glutaraldehyde and stabilize the bond between the glutaraldehyde and the protein. For electrophoresis, the protein was concentrated by precipitation with 5% TCA. The pellet was washed once with water then resuspended in 20 μl of: 2% SDS, 50 mM DTE, 25 mM Tris-Cl (pH 8.8), 10% glycerol, and 0.002% bromophenol blue. After 1 h at 30°C the samples were electrophoresed on the gel system described by Davies and Stark (8). An acrylamide concentration of 3.75% was used. Cross-linked BSA, obtained from Sigma, was used as a mol wt standard.

For electrophoresis of uncross-linked proteins, the gel system of Laemmli (13) was used with a stacking gel of 4% acrylamide and a running gel of 10% acrylamide. Samples were prepared by TCA precipitation as described above, except that immediately prior to loading on the gel they were centrifuged at 100,000*g* for 15 min to remove insoluble material. Gels were stained in 20% methanol, 7% acetic acid, 0.1% Coomassie blue G, 0.2% Coomassie blue R, then destained in 7% acetic acid.

Other Methods. Protein concentrations were determined by Coomassie dye binding using the Bio-Rad reagent with BSA as a standard.

RESULTS AND DISCUSSION

Purification of the ATPase. A 35,000*g* pellet was used as the source of plasma membranes since it has been shown that this fraction is more highly enriched in plasma membranes than is the 80,000*g* microsomal pellet (21). Rather than attempting to purify the plasma membranes further, we chose simply to purify the ATPase directly from this crude membrane mixture. As a first step, the membranes were washed with high salt in the presence of 0.1% Triton X-100. This removed approximately 85% of the total protein giving about a 3-fold purification (Table I). Several detergents were then tested in an attempt to solubilize the ATPase. In contrast to results obtained with the red beet enzyme (19), octylglucoside and deoxycholate, either alone or together, solubilized only a small amount of ATPase activity. This solubilized activity was less sensitive to VO_4 than the activity that remained in the pellet (Table II), indicating that a significant portion of the solubilized activity was not of plasma membrane origin. Analysis of the pellet and supernatant by SDS-PAGE showed that most of the 90 kD band remained with the pellet, whereas a large number of other proteins were released to the supernatant. Together, this indicated that these detergents were solubilizing only small amounts of the plasma membrane ATPase, but were solubilizing significant amounts of other proteins, including some contaminating ATPases. Consequently, the Triton plus KBr washed membranes were routinely extracted with octylglucoside plus deoxycholate as a further purification step.

Table I. *Summary of ATPase Purification*

The yield of protein and ATPase activity at various stages of purification is expressed as the amount obtained per gram fresh weight of starting material. Also given is the specific activity at each stage. All values represent the average of five separate preparations. The protein concentration of the lysolecithin supernatant was not routinely determined since lysolecithin interferes with the Bradford protein assay.

	Protein	ATPase Activity	
		Total	Specific
Crude membranes	0.69[a]	0.65[b]	0.94[c]
Triton/KBr washed membranes	0.10	0.33	3.3
Octylglucoside/deoxycholate extracted membranes	0.031	0.14	4.5
Lysolecithin supernatant		0.084	
Gradient purified protein	0.0034	0.060	17.6

[a] mg/g fresh weight. [b] μmol/min·g fresh weight. [c] μmol/min·mg protein.

Table II. *Recovery of Activity following Octylglucoside and Deoxycholate Treatment*

Triton plus KBr washed membranes were treated with octylglucoside and deoxycholate as described in "Materials and Methods." The various fractions were then assayed for ATPase activity both plus and minus 100 μM Na_3VO_4.

	ATPase Activity		Inhibition by VO_4
	$-VO_4$	$+VO_4$	
	μmol/min		%
Triton/KBr washed membranes	17.1	2.4	86
Octylglucoside/deoxycholate supernatant	3.4	1.4	59
Octylglucoside/deoxycholate pellet	9.7	0.7	93

Solubilization was achieved with lysolecithin at a detergent to protein ratio of 5:1. Under these conditions approximately 60% of the total ATPase activity was released to the supernatant. The amount of protein released to the supernatant could not be determined by the Bradford assay since lysolecithin interferes. However, as can be seen by a comparison of lanes 3 and 4 in the gel shown in Figure 1, only a small fraction of the total protein was solubilized, indicating that in addition to being fairly efficient in solubilizing the ATPase, lysolecithin is also quite specific. Based on a protein determination by a modified Lowry procedure (2), the lysolecithin supernatant had a specific activity of between 10 and 15 μmol/mg·min.

Final purification was achieved by centrifugation on a 20 to 50% glycerol gradient. The peak of ATPase activity occurred at about 37% glycerol. As also noted by Serrano (21) for the oat root enzyme, some inactivation occurs during gradient centrifugation. Addition of phospholipids plus a low concentration of

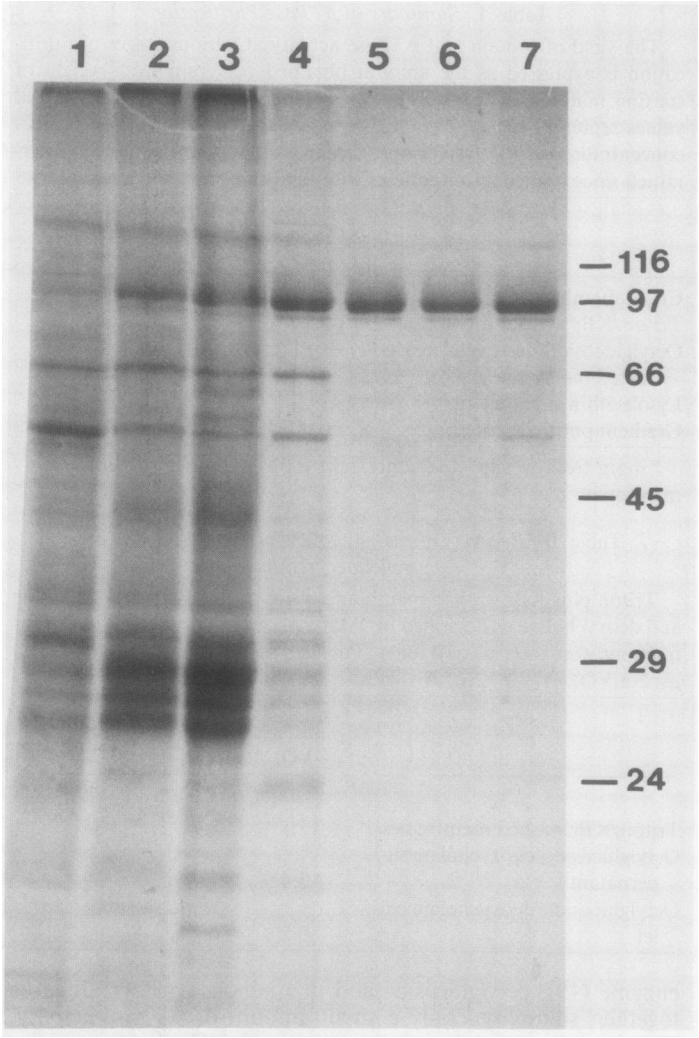

FIG. 1. Protein composition at various stages of purification. Aliquots from various stages in the purification were analyzed by SDS-PAGE using the gel system of Laemmli (11) as described in "Materials and Methods." Lane 1, crude membranes; lane 2, Triton/KBr washed membranes; lane 3, octylglucoside plus deoxycholate extracted membranes; lane 4, lysolecithin supernatant; lanes 5 to 7, three different preparations of gradient purified protein.

detergent to the gradients improved this recovery from about 50 to about 70%. Including ATP had no effect.

A summary based on the average of five preparations is given in Table I. Overall, the yield is about 10%. Since some of the ATPase activity in the crude pellet presumably originates from enzymes other than the plasma membrane ATPase, the overall yield is probably somewhat higher. The protein composition at various stages in the purification is shown in Figure 1. During the course of purification, there is an enrichment in a single band at 90 kD in parallel with the increase in specific activity. At the final stage of purification, the protein is about 80% pure based on densitometer scans.

Comments on the Purification Scheme. Overall, this purification scheme is similar to that described by Serrano (21). Both rely on lysolecithin for solubilization, and final purification is achieved by density gradient centrifugation. There are, nonetheless, several notable differences. In this scheme the protein is already highly enriched before glycerol gradient centrifugation. In fact, the lysolecithin supernatant has a specific activity and purity that is comparable to the most highly purified preparations previously reported. The final gradient centrifugation step does give some additional purification but, unfortunately, does not eliminate all of the contaminating proteins. It is, nevertheless, useful because it separates the protein from the high concentration of lysolecithin used for solubilization and thus makes reconstitution into liposomes possible. At present, we are testing various alternatives to density gradient centrifugation in hope of obtaining a purer final preparation.

Probably the most important factor in the success of this purification is the choice of plant material. Tomato roots contain far more plasma membrane ATPase activity on a fresh weight basis than any other plant source thus far reported. The crude plasma membrane fraction from tomatoes yielded 0.65 units of ATPase activity per gram fresh weight. This compares with values of 0.050 for radish (5), 0.036 for oats (21), and 0.015 for red beets (19).

Properties of the Purified ATPase. The effect of a variety of inhibitors is listed in Table III. A number of compounds including DCCD, DES, VO_4, and F^-, known to inhibit the plasma membrane ATPase from other sources (5, 16, 20, 23), also inhibited the purified tomato root ATPase. The I_{50} for VO_4 inhibition was about 10 μM (Fig. 2), in agreement with previous reports for the partially purified enzyme from other sources (18,

Table III. *Effect of Inhibitors on ATPase Activity*

The purified enzyme was assayed as described in "Materials and Methods" with the addition of various inhibitors at the concentrations indicated.

Addition	ATPase Activity	Percent of Control
	μmol/mg protein·min	
None	19.0	100
100 μM Na_3VO_4	1.6	8
100 μM DCCD	2.2	11
100 μM DES	2.6	13
10 mM NaF	5.2	27
1 mM $NaMoO_4$	16.5	87
5 mM NaN_3	18.1	95
50 mM KNO_3	15.0	79

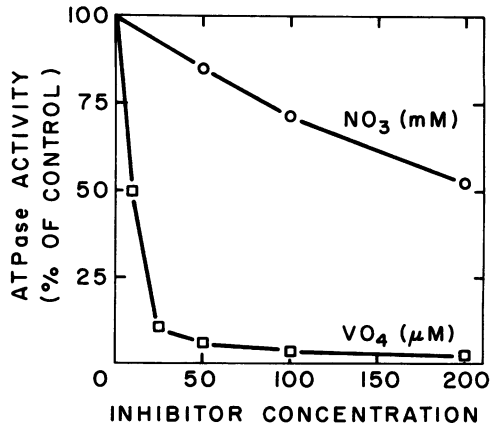

FIG. 2. Inhibition of ATPase activity by nitrate and vanadate. For vanadate inhibition, conditions were as described in "Materials and Methods" with the addition of the indicated concentrations of Na_3VO_4. Nitrate inhibition was measured similarly except that nitrate was added as KNO_3 and the total K concentration was raised to 200 mM in each sample by the addition of an appropriate amount of KCl. Control activity was 17.5 μmol/mg·min for the vanadate curve (50 mM total K) and 17.8 μmol/mg·min for the nitrate curve (200 mM total K), indicating that this increase in K had little effect.

23). A detailed description of the interaction of VO_4 with the ATPase will be presented elsewhere. Surprisingly, activity was also somewhat sensitive to NO_3^- (Fig. 2; Table III). The I_{50} was quite high (>200 mM) indicating that this NO_3^- inhibition was not the result of contaminating tonoplast or mitochondrial ATPases which have I_{50} values of about 10 mM (25). Weak NO_3^- inhibition has often been noted with crude plasma membrane preparations (1, 20) but has generally been dismissed as tonoplast or mitochondrial contamination. As the data here indicates, this may not necessarily be a good assumption. If NO_3^- is to be used as a marker to distinguish between plasma membrane and tonoplast ATPases, one should bear in mind that the two enzymes may differ only in their relative sensitivity to NO_3^-. The best discrimination between the two activities would occur at lower NO_3^- concentrations; concentrations above 50 mM should definitely be avoided.

The pH optimum for ATPase activity was 6.5 (Fig. 3), in agreement with previous reports (5, 20, 23). Activity was stimulated somewhat by K^+, especially at low pH. At pH 5.5 the activity was doubled by 50 mM KCl while at pH 7.5 KCl had virtually no effect. This lack of K^+ stimulation at physiological pH values has been noted by others (9, 23) and tends to raise doubts as to any role for K^+ *in vivo*. The stimulation at pH 6.5 was only about 25%, which is somewhat less than that reported for the partially purified ATPase from other sources (5, 9, 20, 23). This difference is most likely the result of the different plant materials used, although we cannot completely rule out the possibility that our minus-K^+ control is in fact a partially activated ATPase due to its exposure to high K^+ concentrations during the course of the purification. Other monovalent cations also stimulated activity in the order $K^+ > Na^+ > NH_4^+ > Li^+$ (data not shown).

The activity of various partially purified plasma membrane ATPase preparations has been shown to be stimulated by phospholipids and in particular by lysolecithin (5, 21, 23). Consistent with this, the ATPase activity of the tomato root enzyme was strongly stimulated when lysolecithin was added to the assay medium (Table IV). In contrast, the addition of sonicated phospholipids caused significant inhibition. Inhibition by phospholipids was also noted by Serrano (21) with the oat enzyme, prepared by a procedure similar to that used here. Inhibition by phospholipids was completely prevented if the detergent octylglucoside was added with the lipids (Fig. 4). In fact, the activity measured in the presence of lipids plus octylglucoside was actually considerably higher than that measured in their absence. Octylglucoside by itself produced only a small stimulation at low concentrations while at higher levels it was strongly inhibitory. This requirement for lipid plus detergent for maximal stimulation would explain the strong stimulation by lysolecithin since this molecule has both lipid and detergent-like properties.

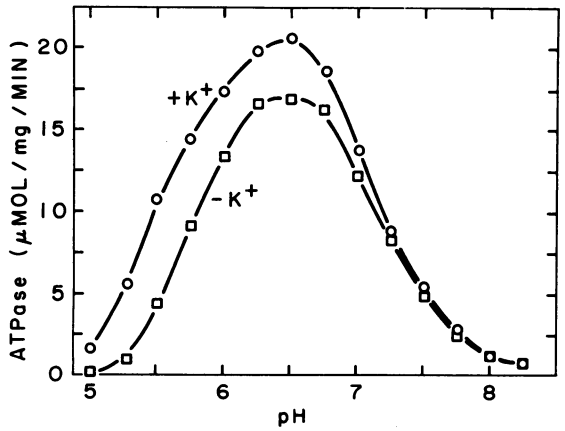

FIG. 3. pH dependence of ATPase activity. Various pH values were obtained by titrating 50 mM Mes with BTP. Where indicated 50 mM KCl was included. Other conditions were as described in "Materials and Methods."

Table IV. *Effect of Lysolecithin and Phospholipids on ATPase Activity*

The purified enzyme was assayed for activity as described in "Materials and Methods" with the additions as indicated.

Treatment	ATPase Activity	Percent of Control
	μmol/mg protein·min	
Control	8.6	100
+ 0.1 mg/ml lysolecithin (Sigma, type I)	16.4	191
+ 0.4 mg/ml phospholipids (Sigma, type IV)	5.0	58

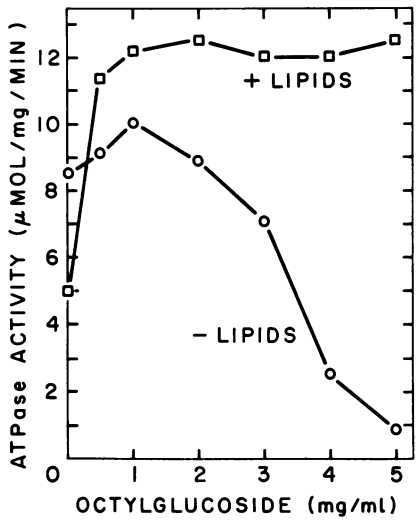

FIG. 4. Effect of octylglucoside on the inhibition by phospholipids. ATPase assays were performed in the presence or absence of 0.4 mg/ml phospholipids (Sigma, type IV) and the indicated concentration of octylglucoside.

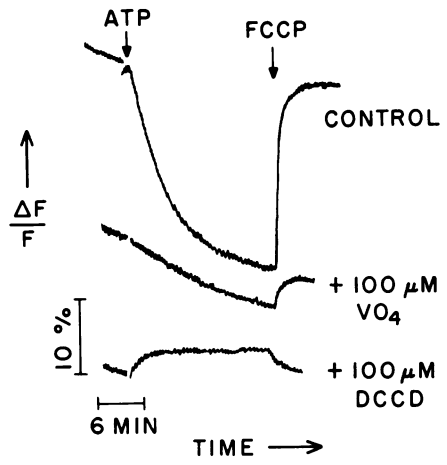

FIG. 5. ATP dependent H^+-transport. The purified ATPase was reconstituted into liposomes and assayed for H^+-transport by quinacrine fluorescence quenching. Arrows indicate where ATP and FCCP were added to final concentrations of 5 mM and 5 μM, respectively. Where indicated, 100 μM DCCD or Na_3VO_4 was added 2 min prior to the ATP.

Reconstitution into Proteoliposomes. To demonstrate that the

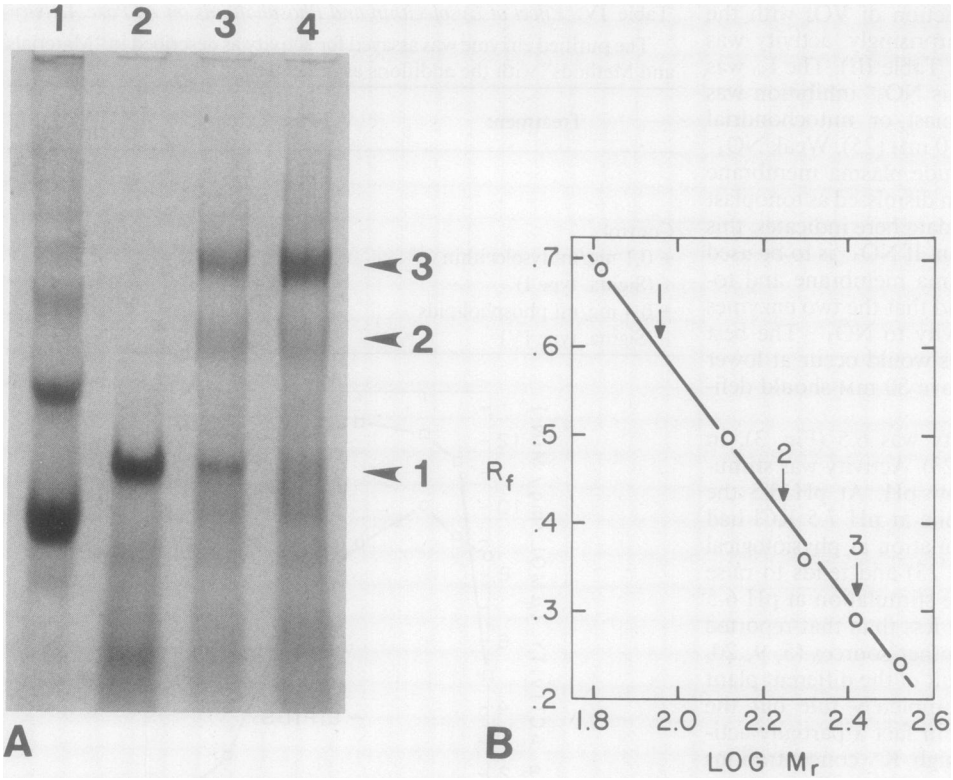

FIG. 6. Electrophoretic analysis of the glutaraldehyde cross-linking products. Cross-linked BSA was used as the mol wt standard; mol wt of the monomer is 66 kD. A, SDS polyacrylamide gel. Lane 1, cross-linked BSA; lane 2, untreated ATPase; lane 3, ATPase cross-linked with 5 mM glutaraldehyde; lane 4, ATPase cross-linked with 10 mM glutaraldehyde. B, Plot of R_F *versus* log M_r for the cross-linked BSA standards. Arrows numbered 1, 2, and 3 indicate the locations of the monomers, dimers, and trimers of the ATPase.

purified ATPase could function as a proton pump the enzyme was reconstituted into proteoliposomes by the freeze-thaw-sonication technique (23). Proton pumping was assayed by measuring quinacrine fluorescence quenching. As shown in Figure 5, fluorescence quenching was dependent on the addition of ATP and could be reversed by the uncoupler FCCP. If either of the ATPase inhibitors VO_4 or DCCD were added prior to the addition of the ATP, little or no fluorescence quenching occurred. These results demonstrate that the reconstituted ATPase does function as a proton pump, in agreement with previous reports for the ATPase purified from oat (21) or partially purified from either radish (6) or red beet (19).

Quaternary Structure. The quaternary structure of the purified protein was examined by cross-linking with glutaraldehyde. This technique has been used successfully with several proteins, including the (Na/K)-ATPase of animal cells (7, 11). If a sufficiently low protein concentration is used little or no intermolecular cross-linking will occur and the products obtained will reflect the aggregation state of the protein prior to the addition of the glutaraldehyde.

In the absence of a glutaraldehyde pretreatment, the purified ATPase electrophoresed as a single band (Fig. 6A, lane 2) with an apparent mol wt of 90 kD (Fig. 6B). Treatment of the protein for 1 h with 5 mM glutaraldehyde led to the appearance of two additional bands (Fig. 6A, lane 3) with apparent mol wt of 175 and 260 kD (Fig. 6B). The mol wt of these bands suggests that they correspond to dimers and trimers of the 90 kD catalytic subunit. When the glutaraldehyde concentration was increased to 10 mM, nearly all of the protein ran in the 260 kD band with no indication of any higher mol wt aggregates (Fig. 6A, lane 4). Increasing the glutaraldehyde concentration to 50 mM did not alter the pattern of products (data not shown), indicating that 10 mM glutaraldehyde is sufficient for complete cross-linking, in agreement with the results of Craig (7). The relative abundances of the various cross-linked products suggest that they are in fact a reflection of the preexisting aggregation state of the protein and are not simply the result of random intermolecular cross-linking between separate monomeric ATPases in solution. Were they the result of intermolecular cross-linking one would have expected a series of bands becoming progressively fainter with increasing aggregation state (as occurs with the BSA standards). Rather, the predominance of trimers following complete cross-linking strongly suggests that the primary form of the enzyme in solution is that of a trimer. The small amount of monomers and dimers that occur even at high glutaraldehyde concentrations could indicate that some heterogeneity in aggregation exists. Such heterogeneity has been shown to occur with the (Na/K)-ATPase (7).

Since the cross-linked products have apparent mol wt which are integral multiples of the catalytic subunit mol wt, it is unlikely that any additional subunits exist. The low mol wt bands seen in the SDS gel of the purified protein (Fig. 1) most likely represent either contaminants or proteolytic degradation products of the catalytic subunit.

While certainly suggestive, the presence of trimers in solution does not, of course, mean that a trimer is necessarily the form found in the plasma membrane *in vivo*. It is, nevertheless, consistent with the radiation inactivation data presented by Briskin *et al.* (4). They showed that the target size for the red beet enzyme was 228,000, or 2.3 times the subunit mol wt. As they point out, this number should be taken as a minimal estimate of the native mol wt; thus, it is more consistent with a trimer than with a dimer. Taken together then, the available data suggests that the ATPase normally exists as a trimer.

Acknowledgment—The authors wish to thank Natalie Bushunow for excellent technical assistance.

LITERATURE CITED

1. BENNETT AB, SD O'NEILL, RM SPANSWICK 1984 H^+-ATPase activity from storage tissue of *Beta vulgaris*. I. Identification and characterization of an anion-sensitive H^+-ATPase. Plant Physiol 74: 538–544
2. BENSADOUN A, D WEINSTEIN 1976 Assay of proteins in the presence of interfering materials. Anal Biochem 70: 241–250
3. BRISKIN DP, RT LEONARD 1982 Partial characterization of a phosphorylated intermediate associated with the plasma membrane ATPase of corn roots.

Proc Natl Acad Sci USA 79: 6922–6926
4. Briskin DP, WR Thornley, JL Roti-Roti 1985 Target molecular size of the red beet plasma membrane ATPase. Plant Physiol 78: 642–644
5. Cocucci MC, E Marrè 1984 Lysophosphatidylcholine-activated, vanadate-inhibited, Mg^{2+}-ATPase from radish microsomes. Biochim Biophys Acta 771: 42–52
6. Cocucci MC, MI de Michelis, MC Pugliarello, F Rasi-Caldogno 1985 Reconstitution of proton pumping activity of a plasma membrane ATPase purified from radish. Plant Sci Lett 37: 189–193
7. Craig WS 1982 Determination of sodium and potassium ion activated adenosinetriphosphatase among the various oligomers formed in solutions of nonionic detergents. Biochemistry 21: 2667–2674
8. Davies GE, GR Stark 1970 Use of dimethyl suberimidate, a cross-linking reagent in studying the subunit structure of oligomeric proteins. Proc Natl Acad Sci USA 66: 651–656
9. Dupont FM, LL Burke, RM Spanswick 1981 Characterization of a partially purified adenosine triphosphatase from corn root plasma membrane fractions. Plant Physiol 67: 59–63
10. Forsberg C 1965 Nutritional studies of *Chara* in axenic cultures. Physiol Plant 18: 275–290
11. Hermann R, R Jaenicke, R Rudolph 1981 Analysis of the reconstitution of oligomeric enzymes by cross-linking with glutaraldehyde: kinetics of reassociation of lactic dehydrogenase. Biochemistry 20: 5195–5201
12. Imbrie CW, TM Murphy 1984 Solubilization and partial purification of ATPase from a rose cell plasma membrane fraction. Plant Physiol 74: 611–616
13. Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680–685
14. Lanzetta PA, LJ Alvarez, PS Reinach, OA Candia 1979 An improved assay for nanomole amounts of inorganic phosphate. Anal Biochem 100: 95–97
15. LeBel D, GG Poirier, AR Beaudoin 1978 A convenient method for the ATPase assay. Anal Biochem 85: 86–89
16. Lew RR, RM Spanswick 1984 Characterization of the electrogenicity of soybean (*Glycine max* L.) roots. ATP dependence and effect of ATPase inhibitors. Plant Physiol 75: 1–6
17. Marrè E, A Ballarin-Denti 1985 The proton pumps of the plasmalemma and the tonoplast of higher plants. J Bioenerg Biomembr 17: 1–21
18. O'Neill SD, RM Spanswick 1984 Effects of vanadate on the plasma membrane ATPase of red beet. Plant Physiol 75: 586–591
19. O'Neill SD, RM Spanswick 1984 Solubilization and reconstitution of a vanadate-sensitive H^+-ATPase from the plasma membrane of *Beta vulgaris*. J Membr Biol 79: 231–243
20. O'Neill SD, RM Spanswick 1984 Characterization of native and reconstituted plasma membrane H^+-ATPase from the plasma membrane of *Beta vulgaris*. J Membr Biol 79: 245–256
21. Serrano R 1984 Purification of the proton pumping ATPase from plant plasma membranes. Biochem Biophys Res Commun 121: 735–740
22. Sze H 1985 H^+-translocating ATPases: advances using membrane vesicles. Annu Rev Plant Physiol 36: 175–208
23. Vara F, R Serrano 1982 Partial purification and properties of the proton-translocating ATPase of plant plasma membranes. J Biol Chem 257: 12826–12830
24. Vara F, R Serrano 1983 Phosphorylated intermediate of the ATPase of plant plasma membranes. J Biol Chem 258: 5334–5336
25. Wang Y, H Sze 1985 Similarities and differences between the tonoplast-type and the mitochondrial H^+-ATPase of oat roots. J Biol Chem 260: 10434–10443