Bot. Bull. Academia Sinica 27: 63-75 (1986)

TWO TYPES OF ELECTROGENIC, H^+-PUMPING ATPases IN MEMBRANE VESICLES FROM SOYBEAN ROOTS

TSUNG-HSIEN CHEN[1] and HEVEN SZE[2]

[1] *Institute of Botany, Academia Sinica*
Taipei, Taiwan 11529, Republic of China
[2] *Botany Department, University of Maryland*
College Park, MD 20742, USA

(Received October 19, 1985; Accepted November 18, 1985)

Abstract

Microsomal membrane vesicles from soybean (*Glycine max* L. cv. Williams) roots were prepared with a 10% dextran cushion and tested for electrogenic H^+-pumping ATPases. The ATP-dependent uptake of $S^{14}CN^-$ which could be reversed through the addition of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) reflected the existence of an ATP-fueled electrogenic H^+ pump which generated positive membrane potentials in these vesicles. Quinacrine fluorescence quenching was induced by MgATP and stimulated by Cl^-. Fluorescence quenching could be reversed by CCCP or gramicidin (H^+ conductors) and provided evidence that an ATP-fueled pH gradient (inside acid) was formed across the membrane of these vesicles. ATP-dependent pH gradient was partially decreased by ADP or AMP, and completely inhibited by N, N′-dicyclohexylcarbodiimide (DCCD). About 30-50% of the pH gradient generated was sensitive to vanadate and the rest was sensitive to nitrate. The vanadate-sensitive ATPase was enriched at 1.16-1.18 g/ml and showed optimal activity at pH 6.3. The nitrate sensitive ATPase was enriched in vesicles of 1.10-1.12 g/ml and showed pH optimum of 8.1. These properties suggest the vanadate-sensitive and nitrate-sensitive H^+-pumping ATPases are associated with the plasma membrane and tonoplast, respectively. K^+ dissipated ΔpH generated by either H^+ pump, indicating that a H^+/K^+ exchange system exists on the plasma membrane as well as the tonoplast.

Key words: ATPase; electrogenic H^+-pump; *Glycine max;* membrane vesicle; quinacrine fluoresence; SCN^- uptake.

Introduction

Electrophysiology studies of intact soybean roots have demonstrated the existence of an electrogenic pump across the plasma membrane (Chen and Spanswick, 1982; Lew and Spanswick, 1984a). To identify the electrogenic pump, we have isolated membrane vesicles from soybean roots and tested for the presence of ATP-driven ion pumps.

Electrogenic H^+ pumps have been demonstrated recently in various plant tissues using isolated membrane vesicles (Sze, 1985). We show here that there are two electrogenic, H^+-pumping ATPases in soybean roots as found in other plant tissues. We also detect a H^+/K^+ exchange system on both the plasma membrane and the tonoplast of soybean roots. Preliminary results of this study have been reported (Chen and Sze, 1984).

Materials and Methods

Plant Material

Soybean seeds (*Glycine max* L. cv. Williams) were soaked in an aerated solution of 0.5 mM $CaSO_4$ for 3 h before incubating in the dark for germination. They were germinated at 25°C between two layers of moistened tissue paper in boxes covered with aluminum foil. After 2 days of growth, the tips (1–1.5 cm) of the roots were harvested.

Preparation of Sealed Microsomal Vesicles

Sealed microsomal vesicles were prepared as described by Sze (1980) with some modifications. Root tips (5–8 g) were homogenized twice with a mortar and pestle in 35 ml of medium containing 250 mM mannitol, 25 mM HEPES-bis tris propane (BTP), pH 7.4, 3 mM ethylene glycol-bis-(β-aminoethyl ether)-N, N'-tetraacetic acid (EGTA), 2 mM dithiothreitol, 0.5% (w/v) bovine serum albumin and 0.5 mM phenylmethylsulfonyl fluoride. After filtration through two layers of Miracloth, the homogenate was centrifuged for 15 min at 13,000 g. The supernatant was centrifuged for 30 min at 60,000 g. The 60,000 g pellet (microsomal fraction) was resuspended in 250 mM mannitol, 2.5 mM HEPES-BTP, pH 7.4 and 1 mM dithiothreitol, layered over a 10% (w/w) dextran cushion prepared in 250 mM mannitol and 2.5 mM HEPES-BTP. After centrifugation for 2 h at 70,000 g, the white interface on top of the dextran cushion was collected and referred as sealed microsomal vesicles.

ATPase Assay

ATPase activity was measured as described (Hodges and Leonard, 1974) and briefly indicated in the Legend to Table 1. Protein was measured by the method of Lowry *et al.* (1951), after precipitation with 10% (w/v) trichloroacetic acid using bovine serum albumin as the standard.

SCN^- Uptake

SCN^- uptake was measured by a filtratlon method described by Sze and Churchill (1981) except the reaction mixture containing 16 μM $S^{14}CN^-$. SCN^- is a

permeant anion and ATP-dependent $S^{14}CN^-$ (Amersham) uptake indicated the generation of an inside positive membrane potential.

Quinacrine Fluorescence Quenching

Quinacrine fluorescence was measured with a Turner model 430 spectrofluorometer by a procedure described by Churchill and Sze (1983) and briefly indicated in the legend to Fig. 2. $MgSO_4$, BTP-Cl or ATP was added to a reaction mixture of 25°C to initate H^+ pumping. The fluorescence of quinacrine is quenched when the amine moves into an acidic compartment (Lee *et al.*, 1982).

Chemicals

Sodium ATP was purchased from Boehringer-Mannheim and converted by Dowex ion exchange and bis-tris-propane titration to ATP-BTP at pH 6.8. Most other chemicals were from Sigma.

Results

ATP-generated Membrane Potential

SCN^- accumulation into the sealed microsomal vesicles was stimulated by ATP and reached a steady state within 5 min (Fig. 1). The enhancement by ATP implied that ATPases on the membrane vesicles generated an inside positive membrane potential and the positive potential provided the energy to drive SCN^- uptake. SCN^- accumulation could be quickly decreased by the protonophore CCCP and this suggested that H^+ might be the electrogenic ion.

Effects of Ionophores and Inhibitors on ATPase Activity

Protonophore CCCP and ionophore gramicidin stimulated ATPase activities

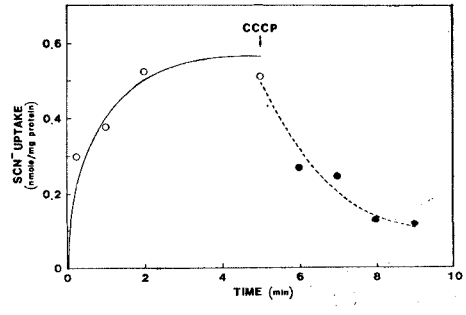

Fig. 1. ATP-induced SCN^- accumulation into microsomal vesicles. CCCP (5 μM) decreased SCN^- uptake. Reaction mixture consisted of 25 mM HEPES-BTP, pH 7.0, 3 mM $MgSO_4$, 16 μM [^{14}C] SCN, 0.4 mg/ml membrane vesicles wtih or without 3 mM ATP.

(Table 1) in these sealed microsomal vesicles which agreed with the idea of an ATP-driven electrogenic H^+ pump. ATP hydrolysis is apparently decreased when an electrochemical gradient is generated and dissipation of the H^+ electrochemical gradient by CCCP or gramicidin releases this inhibition.

Table 1. *Effect of inhibitors and ionophores on ATPase activity in microsomal vesicles from soybean roots*

ATPase activity was determined by measuring Pi release. The reaction mixture consisted of 30 mM HEPES-BTP, pH 6.8, 3 mM $MgSO_4$, 3 mM ATP, 50 mM KCl, 0.5% ethanol plus or minus inhibitors or ionophores

Additions	ATPase activity μmole Pi/mg protein-h (%)
None	11.2(100)
Vanadate (200 μM)	5.2(46.4)
Nitrate (50 mM)	7.8(69.6)
DCCD (10 μM)	4.1(36.9)
CCCP (2 μM)	16.0(142)
Gramicidin (5 μg/ml)	15.8(141)

ATPase activity was inhibited by both vanadate and nitrate which suggested these microsomal vesicles were composed of at least two separate populations of membrane vesicles, presumably from the plasma membrane and the tonoplast (Marin, 1983; Sze, 1985). The majority of the ATPases in membrane vesicles from soybean roots were inhibited by vanadate (Table 1), an inhibitor of ATPase in the plasma membrane (Gallagher and Leonard, 1982). The concentration dependence of the ATPase activities on these two inhibitors are presented in Fig. 2. We have used 200 μM of vanadate or 50 mM of nitrate in all other experiments with these two inhibitors.

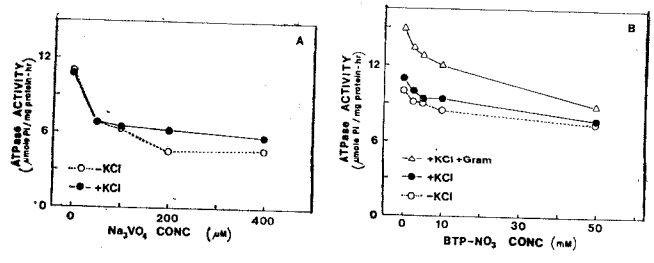

Fig. 2. Vanadate and nitrate inhibition of ATPase activity. Maximum inhibition of ATPase activity by vanadte was reached by 200 μM. Concentration of KCl and gramicidin (Gram) was 50 mM and 5 μg/ml, respectively.

ATP-induced Formation of pH Gradient

Quinacrine fluorescence quenching was used to monitor pH gradient formation in these microsomal vesicles. Percentage fluorescence is a direct measure of quinacrine uptake which depends on the formation of a pH gradient across the membrane (Lee *et al.*, 1982).

Quinacrine fluorescence quenching in these vesicles was induced by ATP (Fig. 3) and this suggested the formation of pH gradient (inside acid) across the membrane of vesicles. DCCD reversed the quenching probably by inhibition of ATPase activity (Table 1); reversal of quenching by CCCP, a protonophore, confirmed the ATP-induced formation of a pH gradient.

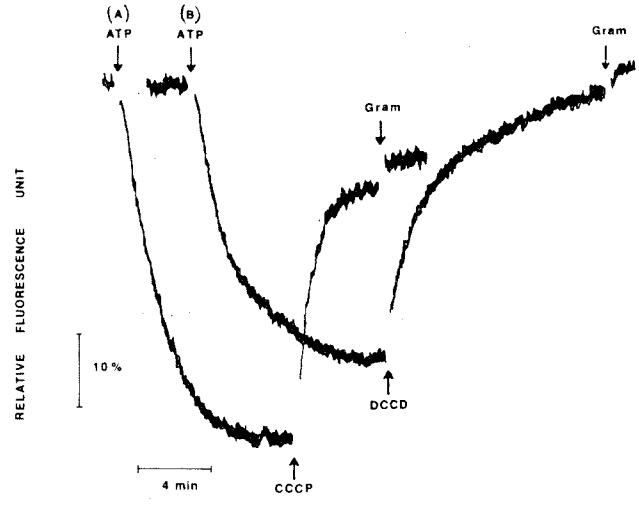

Fig. 3. ATP-induced quinacrine fluorescence quenching in microsomal vesicles from soybean roots. CCCP (5 μM) reversed the quenching. ATP-dependent quenching was decreased by DCCD (50 μM), an inhibitor of ATPase activity (see Table 1), Gram (5 μg/ml gramicidin) completely reversed the quenching. Quinacrine fluorescence quenching was measured in a reaction mixture consisting of 10 mM HEPES-BTP, pH 7.4, 5 mM $MgSO_4$, 50 mM BTP-Cl, 0.33 mM BTP-EGTA, 10 μM quinacrine, 1.5 mM ATP and 0.13 mg/ml membrane vesicles. Exitation and emission wavelengths were 420 nm and 500 nm, respectively.

The ATP dependence was quite specific. Other substrates, such as ADP, AMP or GTP alone could not induce quinacrine fluorescence quenching in these vesicles (Fig. 4). If ADP or AMP was added after a steady state fluorecence quenching was induced by ATP, H^+ pumping was partially inhibited as quenching could be reversed. These results are consistent with the competitive inhibition of ADP or AMP with the substrate, ATP, as shown for the tonoplast-type H^+-ATPase from oat roots (Wang and Sze, in press).

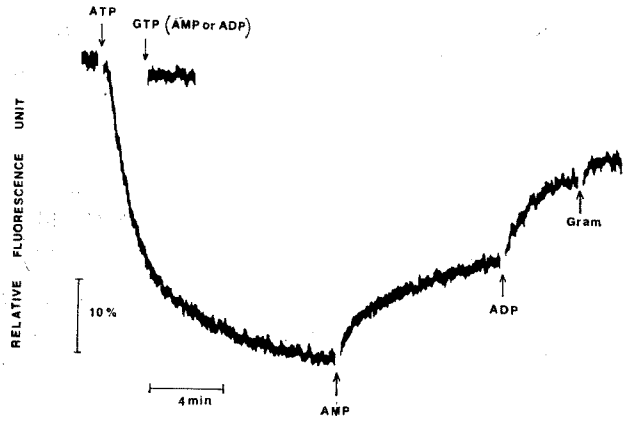

Fig. 4. Quinacrine fluorescence quenching was induced specifically by ATP. ADP, AMP or GTP alone at the same concentration of 1.5 mM had no effect on quinacrine fluorescence. ADP or AMP partially reversed ATP-dependent quenching.

As presented in Fig. 5, ATP-dependent pH gradient formation required Mg^{++} and was stimulated by BTP-Cl. BTP^{+}, a large organic cation, presumably does not move across the membrane; Cl^{-}, a permeable anion, increased pH gradient formation and fluorecence quenching in part by dissipating the membrane potential (Churchill and Sze, 1984).

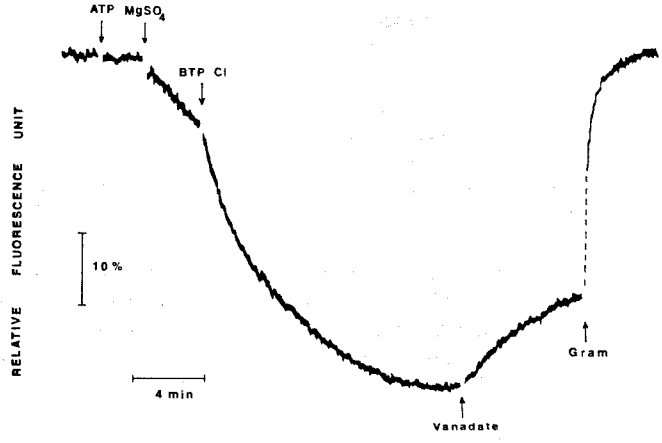

Fig. 5. ATP-induced quinacrine fluorescence quenching was increased by 50 mM BTP-Cl. Vanadate (200 μM) partially decreased the quenching which was completely reversed by 5 μg/ml of gramicidin.

Vanadate and Nitrate Inhibition of pH Gradient Formation

Similar to ATPase activity, quinacrine fluorescance quenching was inhibited

by both vanadate and nitrate. Vanadate at 200 μM could inhibit as high as 50% of the total pH gradient generated (Fig. 6) when vanadate was added before or after addition of Mg-ATP. Since complete inhibition of ATPase activity was reached at a vanadate concentration of 200 μM (Fig. 2), further inhibition of fluorescence quenching by 50 mM nitrate after the addition of 200 μM vanadate (Fig. 7) suggested there were two types of H+-pumping ATPases, one was vanadate-sensitive and one was nitrate-sensive.

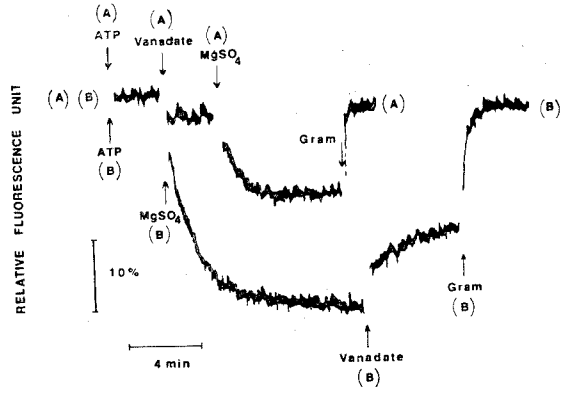

Fig. 6. Vanadate (200 μM) added before (A) or after (B) Mg-ATP reduced quinacrine fluorescence quenching by 50%.

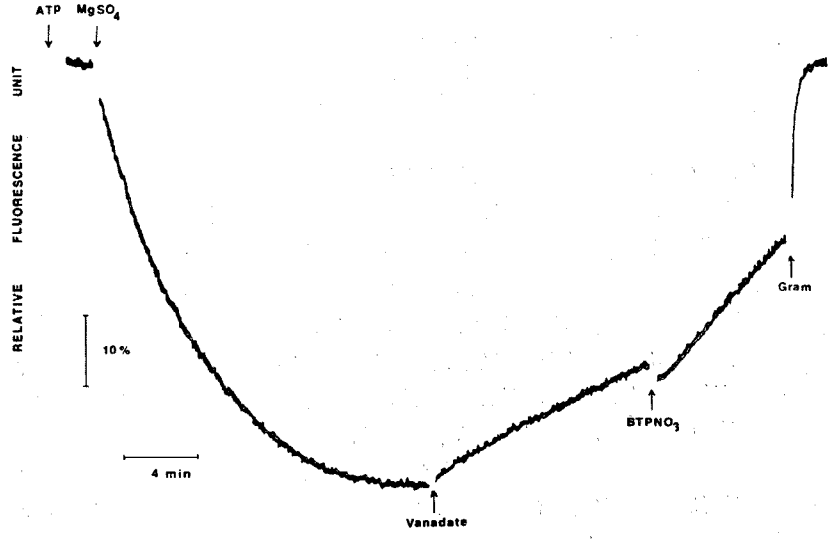

Fig. 7. ATP-induced quinacrine fluorescence quenching was partially inhibited by 200 μM vanadate, BTP-NO_3 (50 mM) further decreased quenching. Gram=5 μg/ml gramicidin.

K^+ Decreased pH Gradient Formation

If K-iminodiacetate (KIDA) was added after fluorescence quenching was induced by Mg-ATP, a decrease in quenching could be observed (Fig. 8) either when the vanadate-sensitive ATPase was inhibited with 200 μM vanadate or when the nitrate-sensitive ATPase was eliminated with 50 mM BTP-NO_3. Since K^+ does not inhibit the ATPase activity (Fig. 2) and IDA^- is imperment, the decrease in fluorescence quenching suggested K^+ collapsed the pH gradient by moving into the vesicles via a H^+/K^+ exchange system. These results are similar to those described for microsomal vesicles from tobacco callus (Sze, 1983). However, pH collapse by K^+ in the presence of vanadate or nitrate suggests that a K^+/H^+ antiport is associated with membranes of both types of H^+-ATPases.

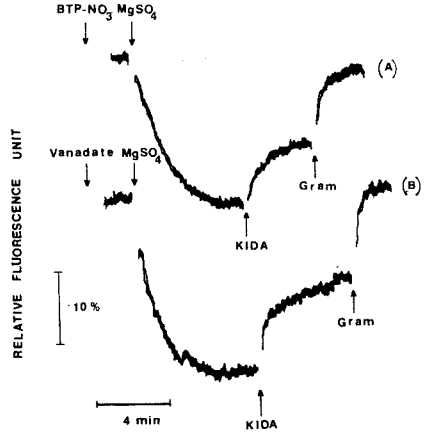

Fig. 8. Mg-ATP induced quinacrine fluorescence quenching was decreased by 50 mM of KIDA (K-iminodiacetate) in the presence of either 200 μM vanadate or 50 mM of BTP-NO_3.

Separation of Two Types of ATPase Activities

Two pH optima could be distinguished when total Mg-ATPase activity was measured. By adding 200 μM of vanadate, we obtained the activity of vanadate-insensitive ATPase with a single pH optimum of 8.1 and by subtracting the vanadate-insensitive ATPase activity from the total ATPase activities, we obtained the activity of vanadate-sensitive ATPase with a single pH optimum of 6.3 (Fig. 9). The nitrate-sensitive ATPase activity (like the vanadate-insensitive ATPase) had the same pH optimum of 8.1 (data not shown).

A linear sucrose gradient was used to separate the microsomal vesicles (Fig. 10). ATPase activity sensitive to vanadate was enriched at a buoyant density of 1.16–1.18 g/ml; activity sensitive to nitrate was greatest at 1.10–1.12 g/ml. Results from both the pH-dependence and sucrose-density gradient centrifugation studies

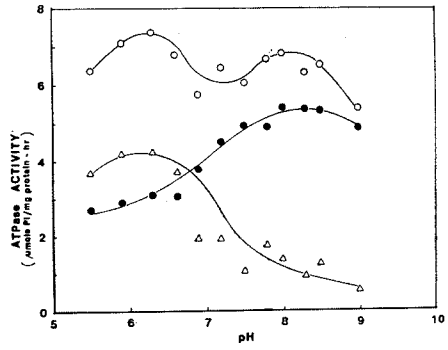

Fig. 9. Effect of pH on ATPase activities. In the absence of vanadate, ATPase activity (○) showed two pH optima. The vanadate-sensitive ATPase activity (△) had a pH optimum of 6.3; the vanadate-insensitive ATPase activity (●) had a pH optimum of 8.1. Vanadate-sensitive ATPase activities were obtained from the difference between ATPase activities in the presence and absence of 200 μM vanadate. Vanadate-insensitive ATPase activities were determined in the presence of 200 μM vanadate. Sodium azide at 0.2 mM and 0.1 mM ammonium molybdate were included in all reaction mixtures to eliminate mitochondria ATPase and unspecific phosphatases, respectively.

suggested the presence of two distinct types of ATPases in two different populations of sealed microsomal vesicles.

Discussion

We have found at least two types of electrogenic H+-pumping ATPases associated with microsomal vesicles isolated from 2-day-old soybean roots. One type is probably located on the plasma membrane, a second type on the tonoplast.

Electrogenicity of these ATPases was concluded from ATP induced SCN^- uptake into microsomal vesicles (Fig. 1). Evidence supporting the H+-pumping capabilities of these ATPases are: (1) SCN^- uptake could be decreased by protonophore CCCP (Fig. 1); (2) ATPase activities were stimulated by CCCP (Table 1) and (3) quinacrine fluorescence quenching could be induced specifically by ATP (Fig. 4) but reversed by DCCD, an ATPase inhibitor, and CCCP (Fig. 3).

The conclusion that there are at least two types of H+-pumping ATPases in these vesicles and their probable association with plasma membrane and tonoplast was based on the following observations: (1) ATPase activity was inhibited by either vanadate or nitrate (Table 1; Fig. 2) which inhibits ATPases from plasma membrane and tonoplast, respectively. (2) ATPase activity sensitive to vanadate was greatest on membranes of 1.16–1.18 g/ml (Fig. 10), a density associated with most other plasma membranes; sensitivity to nitrate was greatest at 1.10–1.12 g/ml,

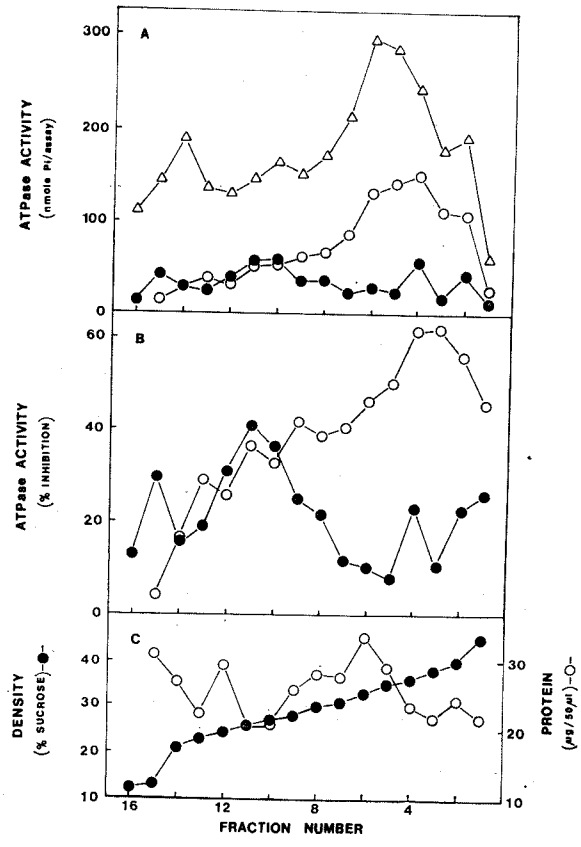

Fig. 10. Distribution of NO_3-sensitive or vanadate-sensitive ATPase in microsomal vesicles separated by a 20-40% linear sucrose gradient.

A. Distribution of nitrate or vanadate inhibition of ATPase activity. ATPase activity was measured in the presence of 50 mM BTP-NO_3 or 200 μM vanadate. Vanadate-sensitive ATPase (determined as in Fig. 9) was enriched at 31-40% sucrose. Nitrate-sensitive ATPase activities were obtained by subtracting ATPase activities in the presence of 50 mM BTP-NO_3 from the total ATPase activites. Sodium azide at 0.2 mM and 0.1 mM ammonium molybdate were included in all the reaction mixtures.

B. Percentage of ATPase activity sensitive to vanadate (○) or nitrate (●). Percent sensitivity to an inhibitor was obtained by dividing the inhibitor-sensitive over the total ATPase activities. ATPase activity associated with membranes of 1.16-1.18 g/ml was 60% or more inhibited by 200 μM vanadate. ATPase activity sensitive to nitrate was greatest on membrane with a buoyant density of 1.10-1.12 g/ml.

C. Protein distribution and sucrose concentration fractioned from a linear sucrose gradient.

a density reported for tonoplasts. (3) Vanadate-sensitive ATPase in these vesicles had a pH optimum of 6.3, close to the value reported for plasma membrane ATPase; vanadate-insensitive ATPase had an optimum of 8.1, a value found several most ATPases of tonoplast origin. Our results are generally consistent with the properties of the plama membrane and tonoplast H^+-ATPases isolated from several other plant tissues (Sze, 1985; churchill *et al.*, 1983; Bennett *et al.*, 1984; Hager and Biber, 1984; Rasi-Caldogno *et al.*, 1985; Mandala *et al.*, 1982; Poole *et al.*, 1984). Our conclusion on the location and inhibitor-sensitivity of these two types of H^+-pumping ATPases generally agrees with the observation of Lew and Spanswick (1984 b).

In some of our experiments, inhibition of ATP-induced fluorescence quenching by vanadate was less pronounced than inhibition by nitrate (Fig. 6), contrary to what should be expected from the greater magnitude of inhibition by vanadate on ATPase activity (Table 1; Fig. 2). Our tentative explanation is that the plasma membrane vesicles were more leaky to H^+ than those from tonoplast.

K^+ decreased ATP-induced quinacrine fluorescence quenching (Fig. 8) in 95% of our experiments. Na-IDA at 50 mM also decreased flourescent quenching with similar magnitude (data not shown). Since K^+ decreased ATP-induced fluorescent quenching in the presence of either 200 μM vanadate or 50 mM nitrate (Fig. 8), H^+/K^+ exchange transport appeared to occur in vesicles from both the plama membrane and the tonoplast. According to the working model, ATP-dependent H^+ pumping would originate from inside-out vesicles of the plama membrane and right-side-out vesicles of the tonoplast (Sze, 1985). If so, the two H^+/K^+ antiport systems would mediate removal of K^+ from the cytoplasm by transport out of the cell across the plasm membrane and across the tonoplast into the vacuole.

Acknowledgements

We thank Ms. Yanzhi Wang for technical assistance. The Turner spectrofluorometer was generously provided by Dr. W. Vanderwoude (USDA, Beltsville). This study was supported in part by National Sciences Foundation Grant (USA; PCM 83-10928) to H. Sze and National Science Council Grant (ROC) to T.-H. Chen.

Literature Cited

Bennett, A. B., S. D. O'Neil, and R. M. Spanswick. 1984. H^+-ATPase from storage tissue of *Beta vulgaris*. I. Identification and characterization of an anion-sensitive H^+-ATPase. Plant Physiol. **74**: 538-544.

Chen, T.-H. and R. M. Spanswick. 1982. The membrane potential of soybean root tip cells. Plant Physiol. **69** (Suppl.): 47.

Chen, T.-H. and H. Sze. 1984. Electrogenic H^+-pumping ATPase in membrane vesicles from soybean roots. *In* S. Seno and Y. Okada (eds.), International Cell Biology 1984. Jpn. Soc. Cell Biol. ISBN 0-12-636780-9, pp. 340

Churchill, K. A. and H. Sze. 1983. Anion-sensitive, H+-pumping ATPase in membrane vesicles from oat roots. Plant Physiol. **71**: 610-617.

Churchill, K. A. and H. Sze. 1984. Anion-sensitive, H+-pumping ATPase of oat roots. Direct effects of Cl^-, NO_3 and a disulfonic stilbene. Plant Physiol. **76**: 490-497.

Churchill, K. A., B. Holaway, and H. Sze. 1983. Separation of two types of electrogenic, H+-pumping ATPases from oat roots. Plant Physiol. **73**: 921-928.

Gallagher, S. R. and R. T. Leonard. 1982. Effects of vanadate, molybdate and azide on membrane-associated ATPase and soluble phosphate activities of corn roots. Plant Physiol. **70**: 1335-1340.

Hager, A. and W. Biber. 1984. Functional and regulatory properties of H+ pumps at the tonoplast and plasma membrane of *Zea mays* coleoptiles. Z. Naturforswch. **35C**: 783-793.

Hodges. T. K. and R. T. Leonard. 1974. Purification of a plasma membrane-bound adenosine triphosphatase from plant roots. Methods Enzymol. **32**: 392-406.

Lee, H. C., J. G. Forte, and D. Epel. 1982. The use of fluorescent amines for the measurement of pH_i: applications in liposomes, gastric microsomes, and seaurchin gametes. *In* R. Nuccitelli and S. W. Deamer (eds.), Intracellular pH: Its Measurement, Regulation, and Utilization in Cellular Functions. Alan R. Liss, Inc., New York. ISBN 0-8451-0305-9, pp. 135-160.

Lew, R. R. and R. M. Spanswick. 1984a. Electrogenicity of soybean roots. Plant Physiol, **75**-1-6.

Lew, R. R. and R. M. Spanswick. 1984b. Proton-pumping activities of soybean (*Glycine max* L.) root microsomes: localization and sensitivity to nitrate and vanadate. Plant Sci. Lett. **36**: 187-193.

Lowry, O. H., N. J. Rosebrough., A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

Mandala, S., I. J. Mettler and L. Taiz. 1982. Localization of the proton pump of corn coleoptile microsomal membranes by density gradient centrifugation. Plant Physiol. **70**: 1743-1747.

Marin, B. 1983. Evidence for an electrogenic adenosine triphosphatase in *Hevena* tonoplast vesicles. Planta **157**: 324-330.

Poole, R. J., D. P. Briskin, Z. Kratky, and R. M. Johnstone. 1984. Density gradient localization of plasma membrane and tonoplast from storage tissue of growing and dormant red beet. Characterization of proton transport and ATPase in tonoplast vesicles. Plant Physiol. **74**: 549-556.

Rasi-Caldogno. F., M. C. Pugliarello, and M. de Michelis. 1985. Electrogenic transport of protons driven by the plasma membrane ATPase in membrane vesicles from radish. Biochemical characterization. Plant Physiol. **77**: 200-205.

Sze. H. 1980. Nigericin-stimulated ATPase activity in microsomal vesicles of tobacco callus. Proc. Natl. Acad. Sci. USA **77**: 5904-5908.

Sze. H. 1983. Proton-pumping adenosine triphosphatase in membrane vesicles of tobacco callus: sensitivity to vanadate and K+. Biochim. Biophys. Acta **732**: 586-594.

Sze, H. 1985. H+-translocating ATPases: advances using membrane vesicles. Annu. Rev. Plant Physiol. **36**: 175-208.

Sze, H. and K. A. Churchill. 1981. Mg/KCl-ATPase of plant plasma membranes in an electrogenic pump. Proc. Natl. Acad. Sci. USA **78**: 5578-5582.

Wang, Y. and H. Sze. 1985. Similarities and differences between the tonoplast-type and mitochondrial H+-ATPases of oat root. J. Biol. Chem. (in press).

由大豆根尖細胞所分離出膜囊上的兩種 Electrogenic, H^+-Pumping ATPases

陳 宗 憲[1] 斯 海 文[2]

1. 中央研究院植物研究所
2. 美國馬里蘭大學植物學系

由大豆根尖細胞所分離出之膜囊爲測試 eletrogenic H^+-pumping ATPase 的材料。由 ATP 所引起的 $S^{14}CN^-$ 進入膜囊作用爲後加入之 CCCP 所逆轉顯示 ATP 所帶動的 electrogenic H^+ 泵使膜囊產生正值電位。MgATP 引起 quinacrine 螢光之尅減，Cl^- 有進一步刺激尅減的作用。因 CCCP 或 gramicidin 均可逆轉 ATP 所引起之 quinacrine 螢光尅減，證實在膜囊內外已產生ATP 所帶動之 pH 梯度分佈（內部較爲酸性）。ADP 或 AMP 可部份抑制 ATP 所造成之 pH 梯度分佈，DCCD 則可完全抑制。所產生之 pH 梯度分佈約有 30–50% 受釩酸根所抑制，其餘部份受硝酸根抑制。受釩酸根所抑制的 ATPase 主要聚於 1.16–1.18 g/ml，最適 pH 爲 6.3；受硝酸根所抑制之 ATPase 聚於 1.10–1.12 g/ml，最適 pH 爲 8.1。這些特性顯示受釩酸根所抑制之 H^+-pumping ATPase 位於細胞膜；受硝酸根所抑制者位於液泡膜。K^+ 可使兩種 H^+ 泵所產生之 pH 梯度分佈消散，顯示在細胞膜及液泡膜上皆有 H^+/K^+ 互換系統存在。