Plant Physiol. (1987) 84, 613–618
0032-0889/87/84/0613/06/$01.00/0

Proton Transport in Plasma Membrane and Tonoplast Vesicles from Red Beet (*Beta vulgaris* L.) Storage Tissue[1]

A COMPARATIVE STUDY OF ION EFFECTS ON ΔpH AND ΔΨ

Received for publication November 17, 1986 and in revised form February 25, 1987

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ABSTRACT

The proton transport properties of plasma membrane and tonoplast vesicles isolated from red beet (*Beta vulgaris* L.) storage tissue were examined and compared. Membrane vesicles isolated with 250 millimolar KCl in the homogenization media and recovered at low density following sucrose density gradient centrifugation displayed characteristics of proton transport (nitrate inhibition, no inhibition by orthovanadate, pH optimum of 7.75, pyrophosphate-driven proton transport) which were consistent with a tonoplast origin. When the KCl in the homogenization medium was replaced by 250 millimolar KI, sealed membrane vesicles were recovered at higher densities in sucrose gradients and displayed properties (orthovanadate sensitivity, no inhibition by nitrate, pH optimum of 6.5) consistent with a plasma membrane origin. A comparison of anion effects (potassium salts) upon ΔpH and ΔΨ revealed a direct correspondence between the relative ability of anions to stimulate proton transport and reduce ΔΨ. For tonoplast vesicles, the relative order for this effect was KI > KBr ≥ KCl > $KClO_3$ > K_2SO_4 while for plasma membrane vesicles, a different order KI > KNO_3 ≥ KBr ≥ $KClO_3$ > KCl > K_2SO_4 was observed. Proton transport in plasma membrane and tonoplast vesicles was inhibited by fluoride; however, plasma membrane vesicles appeared to be more sensitive to this anion. In order to correlate anion effects in the two vesicle fractions with anion transport, the kinetics of anion stimulation of steady-state pH gradients established in the absence of monovalent ions was examined. Anions were added as potassium salts and the total potassium concentration (100 millimolar) was maintained through the addition of K^+/Mes. For plasma membrane vesicles, chlorate and nitrate displayed saturation kinetics while chloride displayed stimulation of proton transport which followed a linear profile. For tonoplast vesicles, the kinetics of chloride stimulation of proton transport displayed a saturable component. The results of this study indicate differences in proton transport properties of these two vesicle types and provide information on conditions where proton transport in the two fractions can be optimized.

The plasma membrane and tonoplast constitute major membrane barriers for nutrient uptake and compartmentation in higher plant cells. Associated with each of these membranes are energy-dependent systems for the primary transport of protons which result in the production of an inwardly directed proton electrochemical gradient across the plasma membrane and an outwardly directed proton electrochemical gradient across the tonoplast (27 and references therein). There is substantial evidence that the mechanism of energy coupling to proton transport involves the action of proton translocating ATPases at each membrane (20 and references therein). In addition, proton transport across the tonoplast can also be energized by a proton translocating pyrophosphatase (24, 25). The proton electrochemical gradients established at each membrane, through primary proton transport, can then serve to drive the secondary transport of other solutes by additional carriers associated with the membranes (15).

Isolated preparations of membrane vesicles have proven to be a useful system for the *in vitro* characterization of transport systems associated with plant membranes (27 and references therein). This work has generally emphasized the study of transport systems associated with the tonoplast since it has proven much more difficult to isolate transport competent vesicles from the plasma membrane. These studies with isolated tonoplast vesicles have allowed the characterization of the nitrate sensitive ATPase associated with this membrane (19, 21, 22), the proton transporting pyrophosphatase (24, 25) and secondary transport carriers for nitrate (2), sucrose (8), sodium (3), and calcium (4, 26). In recent studies, sealed plasma membrane vesicles have been isolated from corn coleoptiles (9), radish seedings (23), and zucchini fruit (17). However, an extensive characterization of the transport properties of these membrane preparations emphasizing comparisons between the plasma membrane and tonoplast has not been carried out.

In a previous report (13), we described a method for the selective isolation of sealed plasma membrane or tonoplast vesicles from the storage tissue of red beet (*Beta vulgaris* L). This method is largely based upon the inclusion of 0.25 M KI or KCl in the homogenization media which results in the production of sealed membrane vesicles derived from either the plasma membrane (KI) or the tonoplast (KCl). This apparent selectivity in the production of sealed membrane vesicles together with the ability to produce membranes in large quantity from the bulky storage tissue of red beet suggest that this system would be useful in the *in vitro* characterization and comparison of transport taking place at the plasma membrane and tonoplast. This study will focus upon these aspects.

MATERIALS AND METHODS

Plant Material. Red beet (*Beta vulgaris* L.) storage roots were purchased commercially. The tops of the plants were removed and the storage roots were stored in moist vermiculite at 2 to 4°C until use. All root tissue used was stored at least 10 d to ensure uniformity in membrane isolation (21).

Membrane Isolation. Plasma membrane and tonoplast vesicles were isolated according to the method of Giannini *et al.* (13).

[1] Supported by United States Department of Agriculture Competitive Grant No. 86-CRCR-1-1977 and a McKnight Foundation Individual Research Grant awarded to D. P. B.

Storage roots were peeled, cut into small squares, and then rapidly placed into a homogenization media containing 250 mM sucrose, 2 mM EDTA, 2 mM Na_2ATP, 1.0% (w/v) BSA (fraction V powder), 0.5% (w/v) polyvinylpyrrolidone (40,000 mol wt), 2 mM PMSF,[2] 15 mM β-mercaptoethanol, 4 mM DTE, 10% (v/v) glycerol, and 70 mM Tris/HCl (pH 8.0). DTE, PMSF, and β-mercaptoethanol were added to the medium just prior to use. In the isolation of plasma membrane vesicles 250 mM KI was included in the homogenization media while in the isolation of tonoplast vesicles, the KI was replaced by an identical concentration of KCl. The tissue was treated with homogenization medium prior to homogenization in a vegetable juice extractor by vacuum infiltration in a 1.5:1 medium:tissue ratio for 5 min at ice temperature.

The storage tissue homogenate was filtered through 4 layers of cheesecloth and then centrifuged at 13,000*g* (8,500 rpm) for 15 min in a Sorvall GSA rotor. The 13,000*g* pellet was discarded and the supernatant was centrifuged at 80,000*g* (32,000 rpm) for 30 min in a Beckman type 35 rotor to obtain a microsomal membrane pellet. The microsomal membrane pellet was suspended in 4 ml of a suspension buffer containing 250 mM sucrose, 2 mM BTP/Mes (pH 7.0), 0.2% (w/v) BSA, 1 mM PMSF (added fresh), 10% (v/v) glycerol, 1 mM DTE (added fresh), and gently homogenized in a dounce type homogenizer. The suspension of membranes enriched for either sealed plasma membrane or tonoplast vesicles was layered over a 25/38% (w/w) discontinuous sucrose density gradient and then centrifuged at 100,000*g* (25,000 rpm) for 2 h in a Beckman SW 28 rotor. The gradient solutions were buffered with 1 mM BTP/Mes (pH 7.0) and contained 1 mM DTE. The membranes present at either the 8/25% (tonoplast) or 25/38% (plasma membrane) interface were removed using a Pasteur pipet. The membranes were either used immediately in transport assays or stored at −80°C after freezing in liquid N_2. In the latter case, full transport activity was retained for up to 3 months.

Optical Measurement of the Vesicle pH Gradient and Membrane Potential. Proton transport in membrane vesicles was measured by the quenching of quinacrine fluorescence (1, 7). The standard assay contained 250 mM sorbitol, 3.75 mM ATP (BTP salt, assay pH), 3.75 mM $MgSO_4$, 2.5 μM quinacrine, 25 mM BTP/Mes (pH 6.5 or 7.75), 50 mM monovalent ions (when present) and 50 to 150 μg of membrane protein. The assay was carried out at pH 6.5 for plasma membrane vesicles and pH 7.75 for tonoplast vesicles. The ionophore reversible quench (Ionophore Reversible; Q/mg protein) was determined following the addition of 5 μM gramicidin D (7). Fluorescence measurements were carried out at 25°C using a Perkin-Elmer Model 203 spectrofluorimeter with the excitation monochronometer set at 430 nm and the emission monochronometer set at 500 nm. Optical measurement of the vesicle membrane potential was carried out under the same conditions as the measurement of the vesicle pH gradient except that 15 μM oxonol V replaced quinacrine in the assay, the excitation monochronometer was set at 590 nm, and the emission monochronometer was set at 650 nm. Any variation in these reaction conditions are indicated in "Results and Discussion."

Protein Assay. Protein was determined by the method of Bradford (5) using BSA as a standard. The Bradford assay reagent was filtered just prior to use.

RESULTS AND DISCUSSION

In previous studies, it was apparent that the inclusion of potassium salts (KI or KCl) at a relatively high concentration (250 mM) in the homogenization medium had a dramatic effect upon the recovery of red beet plasma membrane or putative tonoplast vesicles in a microsomal membrane fraction that were sealed and competent in carrying out ATP dependent proton transport (13). Inclusion of 250 mM KI in the homogenization media resulted in the production of sealed vesicles displaying ATP dependent proton transport inhibited by orthovanadate and migrating to the density expected for plasma membrane on linear sucrose density gradients. Further characterization of the vesicles revealed properties consistent with a plasma membrane origin. Although in our previous study, the inclusion of 250 mM KCl was demonstrated to produce sealed vesicles that displayed nitrate inhibited proton transport and banded at a lower density in sucrose density gradients, further characterization of these vesicles was not carried out. Therefore, it was important to confirm a tonoplast origin for the low density vesicles produced during homogenization of red beet storage tissue in the presence of 250 mM KCl prior to beginning comparative studies.

When the low density vesicles produced in the presence of 250 mM KCl were characterized, properties consistent with a tonoplast origin for the vesicles were found. The corresponding properties of sealed plasma membrane vesicles are shown (for Table I) from our previous work (13) for comparison. Proton transport in the low density fraction was insensitive to orthovanadate but inhibited by nitrate (Table I). In addition, the low density vesicles could use PPi as a substate to drive proton transport. In contrast, the plasma membrane vesicles displayed proton transport which was inhibited by orthovanadate, stimulated by nitrate, and could not be energized with PPi as a substrate. When the effect of assay pH upon proton transport was examined for the low density vesicles, the optimum for transport was broad with peak activity occurring at pH 7.75 (Fig. 1). In contrast, the pH optimum for proton transport with the high density vesicles was sharp with a peak at pH 6.5 (Fig. 1); similar to the pH optimum for plasma membrane ATPase activity (6).

From these results, it is apparent that the low density vesicles display properties consistent with a tonoplast origin (21, 27). In addition to differential sensitivity to nitrate and vanadate, other distinguishing features include the association of PPi driven proton transport and a broader, alkaline pH optimum. These results are similar to those found for tonoplast preparations isolated from a variety of plant species (27 and references therein). For the subsequent comparative studies, transport assays were carried out under optimized conditions with the assay pH for plasma membrane vesicle transport assays at 6.5 and the assay pH for tonoplast membrane vesicle transport assays at 7.75.

The effect of various anions on ΔpH and ΔΨ formation in sealed plasma membrane and tonoplast vesicles was examined

Table I. *Characteristics of Proton Transport in Tonoplast and Plasma Membrane Vesicles Isolated from Red Beet Storage Tissue*

Treatment	Low Density Vesicles		High Density Vesicles	
	%Q/min·mg	*Q/mg*	*%Q/min·mg*	*Q/mg*
Control[a]	62 (100)[b]	152 (100)	61 (100)	181 (100)
Nitrate (100 mM)	19 (31)	28 (18)	65 (107)	202 (112)
Vanadate (100 μM)	62 (100)	166 (109)	16 (26)	36 (20)
−ATP, PPi[c]	22 (35)	55 (36)	0	0

[a] Control assay was carried out in the presence of 250 mM sorbitol, 3.75 mM ATP (BTP salt, assay pH), 3.75 mM $MgSO_4$, 2.5 μM quinacrine, 25 mM BTP/Mes (pH 6.5) (plasma membrane) or 7.75 (tonoplast), 50 mM KCl, and 100 μg of membrane protein. Proton transport was measured by the quenching of quinacrine fluorescence over a 4 min period as described in "Materials and Methods." [b] Values in parentheses refer to the percent of the control assay. [c] PPi replaced ATP in the control assay and was present at 3.75 mM.

[2] Abbreviations: PMSF, phenylmethylsulfonyl fluoride; BTP, Bis-Tris propane.

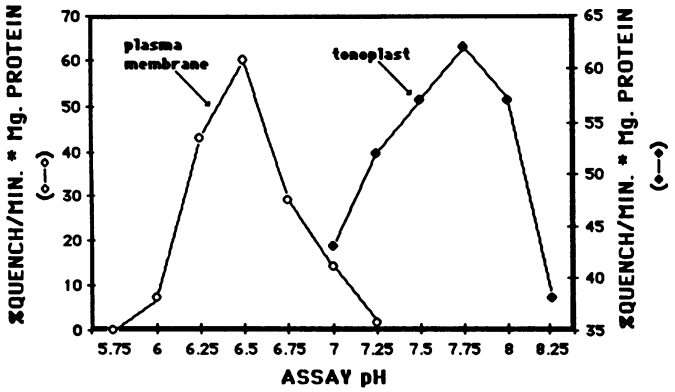

FIG. 1. Effect of pH on proton transport by plasma membrane and tonoplast vesicles from red beet storage tissue. Proton transport was measured at the indicated assay pH by the quenching of quinacrine fluorescence as described in "Materials and Methods."

using fluorescence quenching of ΔpH sensing (quinacrine) and ΔΨ sensing (oxonol V) probes (1, 7). In order to facilitate the presentation of data from a large number of fluorescence quenching traces, the data for proton transport are quantitated both in terms of the initial rate of fluorescence quenching and the final, steady state pH gradient revealed after the addition of 5 μM gramicidin D (for discussion see Ref. 7). The data for ΔΨ production is presented in terms of a steady state potential which was rapidly established (less than 1 min) in plasma membrane and tonoplast vesicles following the addition of Mg:ATP. The potential remained constant for up to 4 min and was related to a baseline determined after the addition of 5 μM gramicidin D. This rapid establishment of a steady state membrane potential in the red beet vesicles differed from the results of Bennett and Spanswick (1) where membrane potentials in tonoplast vesicles from corn roots were measured using oxonol VI. Instead of remaining constant over a short period of time following the addition of Mg:ATP, the initial ΔΨ rapidly declined as the pH gradient in the vesicles increased to a steady state level. Since the measured ΔΨ remained constant over time in this study, the steady state ΔΨ values would represent a reasonable estimate of the initial potential which would be required in comparisons of ΔΨ and ΔpH under various experimental conditions.

The relative order by which anions (50 mM potassium salts) stimulate proton transport in tonoplast vesicles was found to be: KI > KBr ≥ KCl > $KClO_3$ > K_2SO_4 (Table II). This relative order was observed both for quantitation in terms of the rate of fluorescence quenching and the steady state pH gradient. This relative order for anions in stimulating proton transport was identical to the order in which these anions are capable of reducing the vesicle ΔΨ (Table II). These results are similar to what was observed for tonoplast vesicles isolated from sugarbeet storage tissue (7) which indicate that a substantial part of the effect of anions in stimulating proton transport is related to a reduction in the membrane potential through charge compensation. In addition, there is an additional effect of monovalent anions, especially chloride, in the direct stimulation of tonoplast ATP hydrolytic activity (7, 21, 27). When ATPase assays were carried out with the red beet vesicles in the presence of gramicidin D, monovalent anions stimulated activity. However, the level of stimulation was the same for the monovalent anions tested, so that direct stimulation of tonoplast ATPase activity cannot account for the observed relative effectiveness of anions in stimulating proton transport (data not shown). This was similar to what was found in previous studies using sugarbeet tonoplast vesicles (6).

Although the plasma membrane ATPase is directly stimulated by cations (16 and references therein), anions have effects upon

Table II. *Anion Effects upon ΔpH and Δψ in Tonoplast Vesicles from Red Beet Storage Tissue*

Treatment	ΔpH		Δψ
	Initial rate	Ionophore reversible	Ionophore Reversible
	%Q/min·mg	*Q/mg*	*Q/mg*
Control[a]	12.9	4.3	268.9
KI	57.9	125.0	48.6
KBr	55.7	120.7	66.4
KCl	52.1	107.1	75.7
$KClO_3$	28.6	66.4	116.0
K_2SO_4	20.0	42.3	164.3

[a] Control assay was carried out in the presence of 250 mM sorbitol, 3.75 mM ATP (BTP salt, pH 7.75), 3.75 mM $MgSO_4$, 25 mM BTP/Mes (pH 7.75), and 100 to 150 μg of membrane protein. Acid interior pH gradients were measured in the presence of 2.5 μM quinacrine while interior positive membrane potentials were measured in the presence of 15 μM oxonol V, over a 4 min period as described in "Materials and Methods." When potassium salts of anions were tested, the anion concentration was 50 mM.

Table III. *Anion Effects upon ΔpH and Δψ in Plasma Membrane Vesicles from Red Beet Storage Tissue*

Treatment	ΔpH		Δψ
	Initial rate	Ionophore reversible	Ionophore Reversible
	%Q/min·mg	*Q/mg*	*Q/mg*
Control[a]	12	25	1120
KI	136	525	250
KNO_3	102	475	312
KBr	94	438	462
$KClO_3$	75	425	436
KCl	56	212	888
K_2SO_4	36	120	920

[a] Control assay was carried out in the presence of 250 mM sorbitol, 3.75 mM ATP (BTP salt, pH 6.5), 3.75 mM $MgSO_4$, 25 mM BTP/Mes (pH 6.5), and 100 μg of membrane protein. Acid interior pH gradients were measured in the presence of 2.5 μM quinacrine while interior positive membrane potentials were measured in the presence of 15 μM oxonol V, over a 4 min period as described in "Materials and Methods." When potassium salts of anions were tested, the anion concentration was 50 mM.

the proton pumping aspect of this enzyme when present in sealed membrane vesicles (Table III). Anions were found to stimulate proton transport in the following relative sequence: KI > KNO_3 ≥ KBr ≥ $KClO_3$ > KCl > K_2SO_4. As with tonoplast vesicles, the same relative order for stimulation of proton transport by anions was found with respect to the relative ability of anions to reduce the vesicle membrane potential.

From a comparison of results obtained in Tables II and III a number of interesting points can be made. For both membrane systems, KI gives the greatest enhancement of proton transport through charge compensation. In terms of the effectiveness of the monovalent halides tested, the sequence $I^- > Br^- > Cl^-$ was observed for both plasma membrane and tonoplast vesicles. This sequence is similar to the sequence of increasing heats of hydration and decreasing ionic radii (11) so that these parameters may be involved in determining the selectivity of monovalent anion movement, possibly through membrane associated channels (12 and references therein). However, this interpretation is somewhat uncertain since some degree of passive ion conductance may be introduced in the vesicles through damage to the membranes during isolation. Nitrate, which acts to inhibit the tonoplast

proton pumping ATPase, gives substantial stimulation of proton transport in plasma membrane vesicles. Since nitrate inhibited, ATP dependent proton transport is often used to quantitate tonoplast vesicles in isolated membrane preparations, problems may arise if nitrate insensitive proton transport is used as a parameter to quantitate residual proton transport associated with plasma membrane vesicles (9). Under these conditions, the tendency will be to overestimate the relative amount of plasma membrane vesicles since proton transport in these membranes will be stimulated while tonoplast proton transport will be inhibited. For this reason, orthovanadate inhibited, ATP dependent proton transport would appear to be a better quantitative marker for the presence of sealed plasma membrane vesicles (16, 27). Chlorate, an anion chemically similar to nitrate (10), stimulates proton transport in plasma membrane vesicles, but to a much lesser extent in tonoplast vesicles. While this anion has been shown to directly inhibit tonoplast ATPase activity to a lesser extent than nitrate in red beet tonoplast vesicles (measured in the presence of gramicidin D) (14), this apparent inhibition is overshadowed by its effect on reducing $\Delta\Psi$ in the sealed vesicles examined in this study. In contrast, inhibition of the tonoplast ATPase by nitrate is sufficiently strong so that proton transport in the presence of this anion is generally lower than the level observed in the absence of monovalent ions (7, 21). However, it has been shown that nitrate can transiently stimulate proton transport prior to inhibition when added to tonoplast vesicles in which a preexisting pH gradient has been established by ATP-dependent proton pumping (2). As with chlorate, this stimulation was related to a reduction in the vesicle membrane potential with possible transport of nitrate into the vesicle interior (2).

Also of interest is the much lower stimulation of proton transport in plasma membrane by Cl^- than in tonoplast vesicles. While most proton transport assays for tonoplast vesicles are carried out in the presence of KCl, these results suggest that an optimized proton transport assay for plasma membrane vesicles should be carried out in the presence of either KI or KNO_3 to maximize both direct cation effects upon the ATPase (6, 16) and anion effects upon proton translocation.

In order to further characterize these effects for selected ions and to relate their effects on charge compensation to ion movements across the vesicle membrane, proton transport assays were carried out as described by Lew and Spanswick (18). In these assays, vesicles are allowed to generate a steady state $\Delta\mu H^+$ across the membrane which is dominated by $\Delta\Psi$. This is carried out by initiating proton transport with Mg:ATP in the absence of charge compensating monovalent ions. When the fluorescence of the ΔpH sensing probe (quinacrine) reaches a steady state level of quenching, the monovalent ion is added which causes an increase in the extent of fluorescence quenching. As described by Lew and Spanswick (18), this is the result of electrophoretic transport of the ion into the vesicle interior with a concomitant reduction in $\Delta\Psi$ and a corresponding increase in ΔpH. It became apparent during our studies that in order to use this technique to obtain kinetic data (*i.e.* proton transport *versus* ion concentration), a method to control cation concentration was required. This is important since cations such as K^+ directly stimulate the plasma membrane ATPase (16, 20) so that it would be difficult to differentiate direct cation stimulation from anion reduction of $\Delta\Psi$ when using potassium salts. Our approach was to use K^+ salts of selected anions, holding the potassium concentration constant using K/Mes to maximize the stimulation of the plasma membrane ATPase. In addition, it was desirable to use BTP/Cl^- to maintain a constant Cl^- concentration when examining K^+ effects on proton transport. However, prior to these experiments it was important to determine if BTP or Mes had any effect themselves upon proton transport in plasma membrane and tonoplast vesicles.

In order to address this question, experiments were carried out to determine if high concentrations of BTP or Mes (100 mM) affected proton transport in the sealed vesicles. These experiments involved measurements of effects upon both proton pumping and the vesicles membrane potential (data not shown). Transport assays with plasma membrane vesicles carried out in the presence of either 100 mM KCl or 100 mM K/Mes + 100 mM BTP/Cl showed no difference, indicating that these buffers did not affect proton transport or the membrane potential in this system. However, in transport assays using tonoplast vesicles, both proton pumping and the membrane potential were reduced when measured with 100 mM K/Mes + 100 mM BTP/Cl as compared to 100 mM KCl. In order to determine, in this situation, whether BTP or Mes acted to inhibit tonoplast transport, assays where carried out in the presence of 100 mM KCl and either 100 mM Mes (pH 7.75 with BTP) or BTP (pH 7.75 with Mes). Only the assay carried out in the presence of high levels of BTP showed an inhibitory effect upon proton transport. Although the membrane potential was completely collapsed under these conditions, experiments in which anion stimulation of proton pumping was observed revealed no substantial effect of 100 mM Mes.

The kinetics of anion stimulation of proton transport in plasma membrane and tonoplast vesicles is shown in Figure 2. For these experiments, the K^+ concentration was maintained at 100 mM with K/Mes and the anion concentration was varied using K^+ salts. In this way, exposure of the tonoplast vesicles to high levels of BTP was avoided. Chloride stimulation of proton transport in red beet tonoplast vesicles demonstrated a saturable component as observed in soybean tonoplast vesicles (18). In these previous studies, Cl^- was added as KCl so that the similarity in results indicates that K^+ has a minimal effect on tonoplast vesicles. In contrast to the presence of a saturable phase in Cl^- effects with tonoplast vesicles, Cl^- stimulation of proton transport in plasma membrane vesicles increased linearly with increased Cl^- when the K^+ was maintained at 100 mM. This result was surprising and suggested the absence of carrier mediated transport for what would correspond to Cl^- efflux in plant cells. However, previous studies by Rasi-Caldogno *et al.* (23) on the plasma membrane ATPase in vesicles from radish demonstrated KCl stimulation of proton transport which displayed saturation kinetics. In order to further clarify this result, the effect of K^+ concentration on post-steady state ΔpH was examined by maintaining the Cl^- concentration constant at 100 mM using BTP/Cl^- and adding various concentrations of K^+/Mes. Under these conditions, saturation kinetics were observed for the stimulation of proton pumping. Therefore, the results of Rasi-Caldogno *et al.* (23) are most likely related to K^+ effects upon the ATPase rather than Cl^- effects upon charge compensation.

In contrast to the linear stimulation of proton transport by Cl^- in plasma membrane vesicles, NO_3^- and ClO_3^- stimulation of proton transport demonstrated saturation kinetics. The stimulatory effect of K^+ was separated from these anion effects by maintaining all assays at 100 mM K^+ through the addition of K^+/Mes. In addition, a control assay involving the addition of 100 mM K^+/Mes was substracted from each assay to give the stimulation of proton pumping due to anion compensation of the membrane potential. The identical kinetic profiles for these two anions are consistent with their chemical similarity and often similar biochemical effects (10). This profile of saturation kinetics would suggest the involvement of a membrane carrier for nitrate movement corresponding to nitrate efflux in intact plant cells. Attempts were made to measure the stimulation of post-steady state proton pumping in the presence of ClO_3^- with tonoplast vesicles. This was unsuccessful under the conditions of these experiments using $KClO_3$ with K^+ maintained at 100 mM.

Previous studies by Lew and Spanswick (18) have shown that

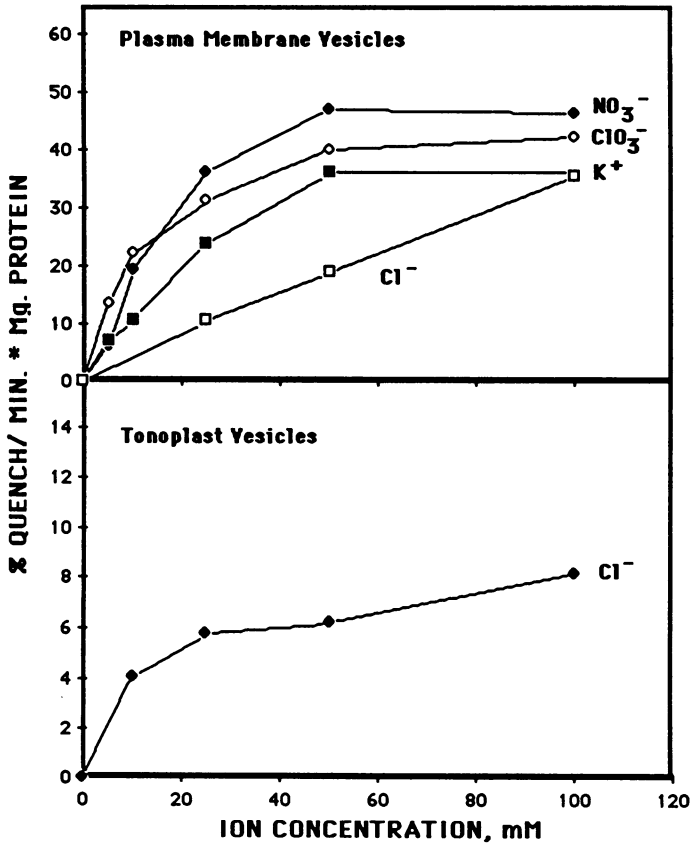

FIG. 2. Ion stimulation of proton transport in membrane vesicles with a pH gradient established in the absence of monovalent ions. Proton transport assays were carried out as described in "Materials and Methods" in the absence of monovalent ions and allowed to reach a steady state level of quinacrine fluorescence quenching. Following the attainment of a steady state pH gradient, 20 μl aliquots of ion stocks were added to yield the indicated final concentration of anions of K^+. The K^+ concentration was maintained at 100 mM in anion stimulation experiments by the addition of K^+/Mes and the rate of quenching observed in the presence of 100 mM K^+/Mes was subtracted from each value to specifically indicate anion stimulatory effects. Potassium stimulation of quinacrine quenching was observed by adding 20 μl aliquots of K^+/Mes stocks and maintaining the Cl^- at 100 mM with BTP/Cl^-.

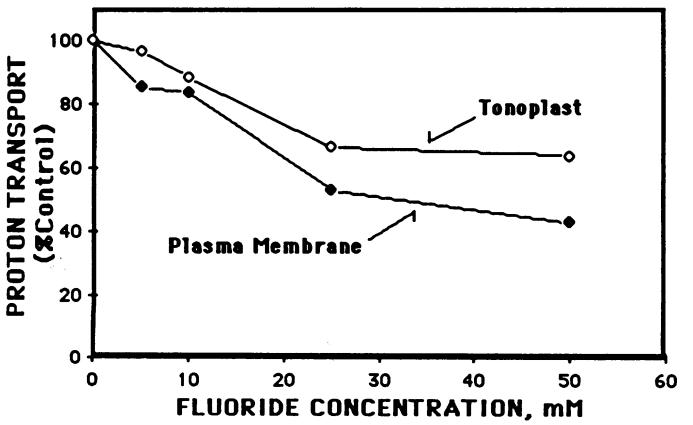

FIG. 3. Fluoride inhibition of proton transport in plasma membrane and tonoplast vesicles. Proton transport was measured as described in "Materials and Methods" in the presence of the indicated concentration of fluoride (as KF). The K^+ concentration was maintained at 100 mM through the addition of K^+/Mes.

plasma membrane and tonoplast ATPase in membrane vesicles from soybean have differential sensitivity to F^- with the plasma membrane ATPase being directly inhibited by this anion. The effects of F^- on proton transport in tonoplast and plasma membrane vesicles of red beet was tested in order to determine whether differential sensitivity to this anion occurs in this plant tissue. In Figure 3, the effect of increasing concentration of F^- (K^+ maintained at 100 mM) on the initial rate of quinacrine fluorescence quenching was examined. Unlike the results presented by Lew and Spanswick (18), proton pumping was inhibited by fluoride in both plasma membrane and tonoplast vesicles preparations. However, the plasma membrane vesicles appeared to be more sensitive to this inhibitor. Since the vesicle preparation method used in this study uses both differential vesicle sealing (KCl *versus* KI in homogenization media) and density gradient centrifugation to produce sealed plasma membrane and tonoplast vesicles free from cross-contamination of transporting vesicles, these results raise question to the proposal that F^- can be used as a specific inhibitor for plasma membrane transport activity.

In conclusion, the results of this study indicate substantial differences in the properties of plasma membrane and tonoplast vesicles with respect to pH optimum for proton transport, sensitivity to buffers, response to ions and the ability to use PPi as a substrate to drive proton transport. These differences may represent characteristic features which could be used to distinguish these vesicle populations. However, a major point demonstrated by this study is the difference in the optimum conditions for proton transport in the two vesicle fractions. Therefore, under the conditions where it is desirable to maximize the measurement of plasma membrane *versus* tonoplast transport activity, separate assay conditions may need to be used for optimal measurement of proton transport.

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