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Salt-induced Na+/H+ antiport in root plasma membrane of a glycophytic and halophytic species of tomato ☆

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Abstract

Plasma membrane and tonoplast vesicles were isolated from roots of both the glycophytic cultivated tomato, ***Lycopersicon esculentum*** (Mill, cv. Heinz 1350) and its halophytic wild relative, ***Lycopersicon cheesmanii*** (Hook, C.H. Mull, ecotype 1401) grown under control and saline conditions. MgATP-dependent proton transport was measured by determining the rate of quench of quinacrine fluorescence.. Rates of quench and rates of ATP hydrolysis were higher for both the plasma membrane and tonoplast from both species when grown under saline conditions. When ATPase activity was measured, the degree of stimulation of ATP hydrolysis in the presence of KCl, NaCl, and choline chloride was similar for the plasma membrane from control and salt grown plants. However, NaCl gave lower rates of proton transport than did KC1 or choline chloride for the plasma membrane of both ***L. esculentum*** and ***L. cheesmanii*** grown under saline conditions. This may be interpreted as evidence of Na+/H+ antiport. A pH gradient (acid interior) was formed in vesicles by adding MgATP. After the establishment of a proton gradient, the effect of cations on proton efflux was estimated by adding EDTA to chelate the Mg^{2+}. Fluorescence recovery rate was used as an indication of the rate of proton efflux. The addition of Na+ enhanced fluorescence recovery compared to K+ in plasma membranes from both species grown under saline conditions. Addition of K+ and valinomycin to the assay media did not affect Na+/H+ exchange, nor did addition of amiloride. No evidence was found for a Na+/H+ antiport mechanism in the tonoplast of either ***L. esculentum*** or ***L. cheesmanii*** regardless of growth conditions.

Keywords: Lycopersicon esculentum; Tomato; H+-ATPase; Plasma membrane; Antiport; Salt tolerance; Salinity

1. Introduction

Salinity is a major cause of crop yield loss in many areas of the world. Although the manifestations of salt stress have been described, the exact mechanisms for their cause remain elusive [1-3]. However, it now appears that the control of ion transport under the high concentrations of NaCl associated with saline stress is crucial to the salt

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Abbreviations: BSA, bovine serum albumin; BTP, 1,3-bis(tris(hydroxymethyl)methyl-amino)propane; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Mes, 2-(N-morpholino)ethanesulphonic acid; ΔpH, pH gradient; PMSF, phenylmethylsulfonyl fluoride; Q, quench; Tris, Tris(hydroxymethyl)aminomethane.

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tolerance of the plant [4] such that limiting net ion transport across the plasmalemma to a rate allowing the cell to adequately compartmentalize Na+ and Cl- in the cytoplasm is critical to salt adaptation [5].

Recent studies have attempted to elucidate the cellular mechansims utilized by the plant to adapt to saline enivronments [5-8]. In our laboratory, we found that Mg^{2+}-dependent ATPase activity in plasmalemma isolated from salt-stressed tomato roots was reduced [9]. In another investigation, we determined that the root cell can modulate the electrostatic properties of the plasmalemma in response to high external salt levels [10]. Modulation of the plasma membrane surface potential by the cell to a more positive value was proposed to have distinct advantages with respect to salt tolerance because a more negative surface would attract fewer cations, thus decreasing their likelihood of transport into the cell. The concomitant attraction of anions to the more electropositive membrane surface might be less harmful than cations as demonstrated by observations on the Na+/Cl- ratios in salt-stressed tomato species [l 1].

Additionally, Na+/H+-antiport systems have been reported by others in a number of salt-tolerant plants and have been suggested to function in conjunction with a membrane-bound H+-ATPase to remove Na+ from the cell interior. For example, Niemitz and Willenbrink [12] and Blumwald and Poole [13] reported a Na+/H+ exchange across the tonoplast. More recently, Guern et al. [14] found evidence in ***Catharanthus roseus*** for Na+/H+ antiport at the tonoplast and Garbarino and DuPont [15] reported NaCl induction of Na+/H+ antiport in tonoplast vesicles from barley roots. With respect to the plasmalemma, the efflux of Na+ across the plasma membrane may also be accomplished by a Na+/H+-antiport system [16]. Na+/H+ antiport has been reported to occur across the plasmalemma of tobacco cells [17], barley roots [18,19], red beet [19], and the halophyte ***Atriplex nummularia [20].*** In algae, ***Chara corallina*** [21] and the green halotolerant alga, ***Dunaliella [22,23],*** Na+/H+ antiport activity has been reported at the plasmalemma.

In tomato, there is evidence that a Na+/H+ antiport mechanism may be operating in root tissue. Mennen et al. [24] measured the effect of an artificial pH gradient on Na+ uptake by intact ATP-depleted tissue. They reported evidence for the operation of a Na+/H+ antiport at the plasmalemma and tonoplast of ***Lycopersicon esculentum*** as well as other species. As a consequence, the present study was designed to determine if Na+/H+ antiport activity is detectable in isolated membrane fractions of tomato roots enriched in plasmalemma or tonoplast and if salinity stress would induce its activation or synthesis. Plasma membrane-enriched and tonoplast-enriched vesicle fractions isolated from both the salt-sensitive cultivated tomato, ***Lycopersicon esculentum, cv.*** Heinz 1350 and its salt-tolerant wild relative ***Lycopersicon cheesmanii,*** ecotype 1401, were examined. By utilizing the membrane-bound H+-ATPase to generate a pH gradient [25], evidence for a Na+/H+ antiport was found in plasma membrane vesicles isolated from salt-exposed roots of both tomato species. However under nonsaline conditions, neither the glycophytic cultivated tomato nor the halophytic wild tomato grown showed this capacity. In addition, Na+/H+ antiport activity in the tonoplast was not evident. In conclusion, the results presented characterize a physiological determinant of salt adaptation and link salt adaptation to a plasma membrane Na+/H+ antiport.

2. Materials and methods

2.1. *Plant material*

Seeds of two tomato genotypes, ***Lycopersicon esculentum*** (Mill, cv. Heinz 1350) and ***Lycopersicon cheesmanii*** (Hook, C.H. Mull, ecotype 1401), were germinated in vermiculite for 2 weeks and 4 weeks, respectively, and then grown in solution culture containing nutrient solution (0.5 × Hoagland solution, pH 6.0) in a controlled-environment chamber. The day/night temperatures were maintained at 30/25°C during the 12-h day length cycle. One week after transplanting, 25 mM total salt (5:1 molar ratio NaCl and $CaCl_2$) was added each day until the desired final salt treatment of 50, 75, or 100 mM was obtained. Roots were harvested 3 weeks after final treatment concentration was attained.

2.2. Plasma membrane isolation

Plasma membrane and tonoplast-enriched membrane fractions were isolated on sucrose step-gradients as described by Garbarino and DuPont [15]. Briefly, excised tomato roots were rinsed with deionized water, blotted to remove excess water and homogenized in buffer containing 250 mM sucrose, 30 mM Tris, 8 mM EDTA, 4 mM DTT, 2 mM ATP, 1 mM PMSF, 0.5% BSA, and 0.1% β-mercaptoethanol, pH 7.8. The homogenate was filtered through four layers of cheesecloth and centrifuged for 20 min at 10 000 x g. The resulting supernatant was then centrifuged at 85 000 *x g* for 40 min. The microsomal pellet was first resuspended in a buffer consisting of 10 mM Tris-Mes (pH 7.8) with 250 mM sucrose, 2.0 mM DTT, 5 $\mu g \cdot ml^{-1}$ chymostatin, and then layered on a step-gradient made up of 9 ml each of 22,30, 34, and 40% (w/w) sucrose containing 4 mM DTT, 2.5 mM EDTA, 10 mM Tris (pH 7.8 with tetramethylammonium hydroxide). The gradient was centrifuged at 100 000 x g for 2 h. The tonoplast-enriched fraction was collected at the 22/30% interface and the plasma membrane-enriched fraction was collected at the 34/40% interface. Samples were used either immediately or stored in liquid nitrogen and used within 2 weeks.

2.3. Measurement of ATPase activity and ΔpH

ATPase hydrolysis assays were performed in 25 mM BTP-Mes (pH 7.5) buffer containing 3.75 mM $MgSO_4$, 3.75 mMTris-ATP, 0.5 mM $(NH_4)_6Mo_7O_{24}$, and 0.005% (w/v) lysolecithin. Salts (50 mM) were added as indicated. The assay temperature was maintained at 30°C for plasma membranes and 21°C for tonoplast membranes. Linearity was checked over the time course of the assay (30 min). Boiled controls were run to determine background phosphate. Protein concentrations were determined using the calorimetric assay of Peterson [26]. Inorganic phosphate was measured by the method of Peterson [27]. Plasma membrane-bound ATPase activity was determined by the difference in activity between assays supplemented with 50 mM salts or 50 mM salts plus 100 μM sodium orthovanadate [28].

The pH gradient formation experiments were performed using the method of Suhayda et al. [10] with some modification. Proton transport activity was measured in the presence of different salts by the quenching of the fluorescent probe, quinacrine, using a SLM 8000 spectrofluorometer. Excitation and emission wavelengths were 430 and 500 nm, respectively. The membrane vesicles were assayed in a buffer consisting of 150 μg of membrane protein in 250 mM sorbitol, 25 mM BTP-Mes (pH 7.5), 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), 2.5 μg quinacrine, 1 $mg \cdot ml^{-1}$ fatty acid-free BSA, and 50 mM monovalent ions. Assays of plasma membranes vesicles were conducted at 30°C and those of tonoplasts at 21°C.

Experiments on the effects of Na+ and EDTA on the rate of dissipation of the ATP-generated pH gradient were performed essentially as described by Garbarino and DuPont [15]. The assay buffer contained 150 μg of membrane protein in 250 mM sorbitol, 25 mM BTP-Mes (pH 7.5 for plasma membrane and pH 7.8 for tonoplast), 3.75 mM $MgSO_4$, 3.75 mM ATP (BTP salt), 2.5 μM quinacrine, 1 mg. ml^{-1} fatty acid-free BSA, and 50 mM monovalent ions (KNO_3 for plasma membrane and choline chloride for tonoplast). The assay temperature was maintained at 30°C for plasma membrane and 21°C for tonoplast. After a pH gradient was formed by the addition of 3.75 mM MgATP, 8 mM Tris-EDTA was added in order to complex Mg^{2+} and stop H+-pumping. Proton leakage was monitored by the increase in fluorescence.

3. Results

3.1. Purity of membrane-enriched vesicle fractions

The purity of membrane-enriched vesicle fractions isolated from discontinuous sucrose step gradients were assayed using inhibitors specific for mitochondria (azide), plasma membrane (vanadate) and tonoplast (nitrate) ATPase activity as well as non-specific phosphatase (molybdate) activity (Table 1). The plasma membrane-enriched fraction was collected at the 34140% interface and ATPase activity in this fraction was found to be highly sensitive to vanadate (78% inhibition). ATPase activity in the tonoplast-enriched fraction, collected at the 22/30% interface, was relatively insensitive to vanadate (13% inhibition) but highly

Table 1
Inhibitor sensitivity of *L. esculentum* tomato root plasma membrane- (PM) and tonoplast- (TP) enriched fractions isolated on step sucrose gradients (nonsalinized, control plants)

Assay additions	ATPase activity (μM P·mg protein^{-1}·h^{-1})	
	Plasma membrane	Tonoplast
Mg-ATP only	51.2	46.3
+ 50 mM KCl	55.5 (108%)	50.8 (110%)
+ 50 mM KCl + 1 mM Mo + 1 mM Azide	34.2 (62%)	39.4 (85%)
+ 50 mM KCl + 100 μM Vanadate	11.3 (22%)	40.0 (87%)
+ 50 mM KCl + 50 mM KNO_3	53.4 (104%)	0.4 (0.1%)

ATPase hydrolysis assays were performed in 25 mM BTP–Mes (pH 7.5) buffer containing 3.75 mM $MgSO_4$, 3.75 mM Tris–ATP, 0.5 mM $(NH_4)_6Mo_7O_{24}$, and 0.005% (w/v) lysolecithin. Numbers in parentheses indicate percent of Mg-ATP activity.

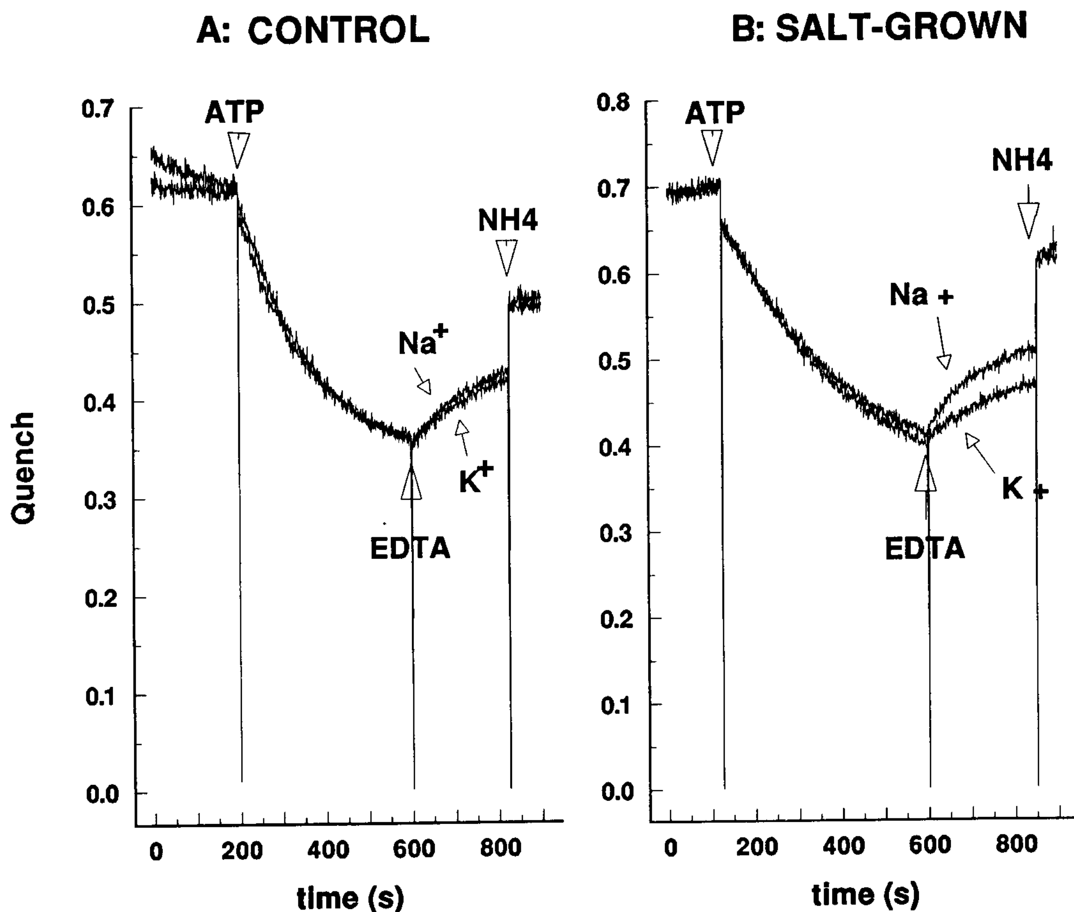

Fig. 1. Effect of Na^+ and EDTA on the rate of dissipation of the ATP-generated pH gradient in plasma membranes isolated from control (A) and 50 mM salt-grown (B) tomato roots (*L. esculentum*, cv. Heinz 1350). Experimental procedures were as described in Section 2, Materials and Methods. Concentrations of 3.75 mM ATP (BTP salt) and 10 mM NH_4Cl were added as indicated by the appropriate arrows; 8 mM EDTA plus 30 mM Na-gluconate or K-gluconate were added at the times indicated by the arrows.

sensitive to nitrate. The data presented in Table 1 represent values obtained from membranes isolated from roots of nonsaline-treated (control) ***L. esculentum.*** ***No*** significant differences in inhibitor sensitivity were detected due to species or salt treatment. As found in the cultivated tomato, MgATP-dependent H^+-transport activity in ***L. cheesmanii*** plasma membranes displayed a broad pH dependence with an optimum near 7.5 (Ref. [9]; present data not shown).

3.2. *ΔpH* ***Generation in control and salt-stressed plasma membrane vesicles***

Salt stress increased MgATP-dependent formation of a pH gradient in plasma membrane vesicles as measured by quinacrine quench assay (Fig. 1A,B). It is commonly believed [25,29] that the MgATP-dependent, curvilinear quenching of quinacrine fluorescence represents a two-component system consisting of initial quench rate and the steady-state quench. The quench curves obtained from plasma membrane-enriched vesicles of ***L. cheesmanii*** showed that during **ΔpH** formation, salt stress increased quench rates as measured either 1 min (Q_1) or 4 min (Q_4) after MgATP addition (Table 2). It is clear from Fig. 1 that the quench rate is linear up to 1 min. We have included the 4-min time-point for comparison. Quench rates (Q,) were calculated on the basis of NH_4Cl reversible quench.

In order to demonstrate salt-stress dependent increases in **ΔpH** formation in both the cultivated and wild species, a series of plant growth experiments and membrane isolations were replicated four times. We found that the magnitude of increase in **ΔpH** formation was greater in the wild species (+33%) than in the cultivated species (approximately +28%), and was more consistently found to be significant within a given experiment in the wild species.

3.3. ***Effect of Na+ on generation of pH gradient***

Work by Mennen et al. [24] indicated that a Na^+/H^+ antiport mechanism may be operating in tomato roots. Initial measurements in this study were made to examine the relative effect of KCl, NaCl, and choline chloride on the generation of a pH gradient by the H^+-ATPase. In plasma membrane vesicles of both species, KC1 stimulated H^+-pumping to a slightly, but significantly greater degree, than choline chloride regardless of whether the vesicles were derived from control or salt-

Table 2
Quench rates (**ΔpH**) after 1 min (Q_1) and 4 min (Q_4) in plasma membrane vesicles isolated from control and salt-stressed (75 mM salt) tomato species

Experiment	*L. esculentum*		*L. cheesmanii*	
	Q_1	Q_4	Q_1	Q_4
Control				
1	11.95 (1.93)	9.16 (0.49)	10.09 (0.84)	8.39 (0.34)
2	12.98 (2.01)	9.37 (0.25)	12.09 (0.09)	9.33 (0.15)
3	12.47 (0.61)	11.56 (0.79)	13.63 (0.62)	11.62 (0.27)
4	12.77 (0.47)	11.41 (0.36)	13.66 (0.86)	11.01 (0.21)
Average (SD.)	12.54 (0.39)	10.37 (1.11)	12.37 (1.6)	10.09 (1.29)
Salt				
1	12.63 (0.30)	10.16 (0.01)	15.38 (0.01)	12.06 (0.09)
2	16.89 (2.04)	12.78 (0.25)	16.71 (0.30)	13.93 (0.03)
3	12.83 (0.95)	13.33 (0.10)	17.41 (1.24)	14.47 (0.21)
4	15.42 (0.38)	14.69 (0.07)	16.06 (0.20)	13.11 (0.32)
Average (SD.)	14.44 (1.79)	12.74 (1.64)	16.39 (0.75)	13.39 (0.91)

Assays included 50 mM KNO_3. Q_1 calculated as decrease in relative fluorescence (quench) 60 s after addition of ATP. Q_4 calculated as average decrease in fluorescence per minute after 4 min. Values in parentheses indicate standard deviations from the mean calculated over three to four assays or from four experiments.

grown roots. Conversely though, proton gradient formation was less in the presence of NaCl than when KC1 or choline chloride was present even though Na+ stimulated Mg-ATP hydrolysis to a slightly greater degree than K+ (Table 3). Since the differences in quinacrine quench rates cannot be attributed to differences in ATP hydrolysis, it is possible that the observed reduced proton gradient formation is the result of an exchange of Na+ and H+.

3.4. *Effect of Na+ on dissipation of ATP-generated pH gradient*

To test whether a Na+/H+ antiport mechanism was present in the plasma membrane, the effect of Na+ on the dissipation of a transmembrane pH gradient was investigated. A pH gradient across the membrane vesicles was generated by using the activity of the membrane-bound ATPase in a manner similar to Garbarino and DuPont [15]. Proton pumping was initiated by adding 3.75 mM BTP-ATP in the presence of 3.75 mM $MgCl_2$. The ATPase was subsequently inhibited by adding EDTA to chelate Mg^{2+} and proton efflux from the vesicles was monitored by following the recovery of fluorescence (Fig. 1). In the absence of a permeable counter ion, the recovery of fluorescence is low and attributed to passive leakage from the vesicles. In the case of plasma membrane vesicles from nonsalinized control roots of either species, the addition of 30 mM Na-gluconate after EDTA did not result in an increase in the recovery of fluorescence when compared to an equal amount of K-gluconate (Table 4). When 8 mM EDTA was added alone, the fluoresecence trace was identical to 8 mM EDTA plus 30 mM K-gluconate (data not shown). However, when the same experiment was performed on vesicles isolated from roots of salt-stressed plants, the addition of Na+ resulted in an increase in the recovery of fluorescence compared to K+ (Fig. 1B; Table 4).

As pointed out by Blumwald and Poole [13], the dissipation of the pH gradient may be due to Na+ diffusing down its chemical gradient resulting in an increase in the membrane potential and an increase in the passive efflux of H+ through conductive pathways in the isolated plasma membrane vesicles. To test this possibility, we added 1 μM

Table 3
Cation effects on proton gradient formation (%Q $\cdot min^{-1}$) and on the hydrolysis activity (μM P $\cdot$ mg protein-' $\cdot h^{-1}$) of ATPase in tonoplast vesicles isolated from roots of cultivated and wild tomato plants subjected to nonsaline conditions and two salinity levels, 50 mM and 100 mM salt

Species	Additions to medium	Plant growth conditions		
		Control	50 mM	100 mM
Proton gradient formation (%Q $\cdot$ min^{-1})				
L. esculentum	Choline Cl	7.58 (0.07)	13.22 (0.15)	8.00 (0.41)
	KC1	8.33 (0.23)	13.84 (0.12)	8.06 (0.23)
	NaCl	7.65 (0. IO)	9.79 (1.14)	6.28 (0.23)
L. cheesmanii	Choline Cl	5.97 (0.49)	8.09 (0.37)	7.79 (0.21)
	KCI	6.59 (0.02)	9.74 (0.26)	8.79 (0.05)
	NaCl	5.64 (0.42)	6.80 (0.20)	6.07 (0.51)
Hydrolysis activity (μM P $\cdot$ mg protein-' $\cdot h^{-1}$)				
L. esculentum	Mg only	43.8 (0.54)	87.7 (0.83)	74.8 (2.75)
	KC1	52.9 (5.59)	92.5 (2.55)	85.7 (2.99)
	NaCl	53.1 (1.23)	100.8 (3.23)	100.3 (4.93)
L. cheesmanii	Mg only	50.1 (1.60)	94.8 (1.27)	98.5 (6.44)
	KC1	52.6 (1.09)	101.4 (1.85)	121.2 (2.49)
	NaCl	62.2 (0.54)	108.5 (1.41)	113.5 (2.42)

Numbers in parentheses represent standard deviations from the mean. See Section 2, Materials and methods for assay conditions and procedures.

Table 4
Rate of recovery of fluorescence quench

Membrane	Treatment	Quench recovery” ($\%Q \cdot min^{-1}$)		
		$Q_{1\ min}$	$Q_{2\ min}$	$Q_{3\ min}$
L. esculentum				
Control	+K	-10.42 (0.43)	-8.33 (0.02)	-7.15 (0.30)
	+Na	-9.61 (0.41)	-7.73 (0.25)	-6.64 (0.18)
Salt	+K	-25.32 (1.42)	-21.30 (1.12)	-18.06 (1.67)
	+Na	-37.03 (2.20)	-29.16 (0.58)	-23.96 (0.07)
L. cheesmanii				
Control	+K	-13.00 (0.39)	-10.91 (0.12)	-8.95 (0.42)
	+Na	-15.60 (1.59)	-12.59 (0.91)	-10.95 (1.08)
Salt	+K	-19.20 (0.62)	-16.09 (0.33)	-14.67 (0.63)
	+Na	-33.23 (2.17)	-26.38 (I .64)	-21.85 (0.56)

Quench recovery was measured from 1 to 3 min following the addition of 8 mM Tris-EDTA followed by 30 mM K-gluconate (+K) or Na-gluconate (+Na) as an indication of Na+/H+ antiport activity in plasma membrane vesicles isolated from control and salt-stressed tomato roots. Membranes were isolated from roots grown in 0.5 x Hoagland’s solution, pH 6.0 without salt (control) or with added salt, 50 mM, 5:l molar ratio NaCl and $CaCl_2$ (salt). Results of two experiments are reported. Numbers in parentheses indicate standard deviations from the mean.
“Rate of recovery upon the addition of 30 mM Na-gluconate or K-gluconate.

valinomycin to the assay buffer [15]. This addition did not eliminate the Na+-induced fluorescence recovery (Fig. 2) further supporting the presence of a Na+/H+ antiport mechanism in the plasma membrane of salt-stressed roots.

3.5. *Effect of amiloride on Na+/H+ exchange*

Amiloride has been used as a specific inhibitor of Na+/H+ exchange in animal membranes [30-32], sugar beet tonoplast vesicles [13], and in ***Dunaliella*** plasma membrane [22]. Approximately 3 min after the addition of BTP-ATP, 0.5 mM amiloride was added to tomato plasma membrane-enriched vesicles. After an additional 40 s, EDTA and either K-gluconate or Na-gluconate was added. The addition of amiloride had little effect on Na+/H+ exchange (Fig. 2).

3.6. *Effect of Na+ on dissipation of ATP-generated pH gradient in tonoplast vesicles*

A proton gradient was established in tomato tonoplast vesicles by adding 3.75 mM BTP-ATP in the presence of 3.75 mM $MgCl_2$ and 50 mM choline chloride. The presence of a Na+/H+ exchange mechanism was assayed in the same manner as with plasma membrane vesicles. No clear evidence of a Na+/H+ antiport mechanism was found in tonoplast of either ***L. esculentum*** or ***L. cheesmanii.*** Salt treatment did not change this response. For example, in tonoplast fractions isolated from control roots of ***L. esculentum,*** the rate of fluorescence recovery after the addition of K-gluconate and EDTA was $4.37\%Q \cdot min^{-1}$. When 30 mM Na-gluconate replaced K-gluconate, the rate of fluorescence recovery was $4.24\%Q \cdot min^{-1}$. In vesicles from salt-stressed roots, the values were 2.96 and $2.59\%Q \cdot min^{-1}$, respectively.

4. **Discussion**

Previous work at this laboratory with the cultivated tomato, ***L. esculentum,*** could not demonstrate that 75 mM salt-stress significantly changed ΔpH formation [10]. The ability to detect differences in these studies may be attributed to the use of a threefold greater protein concentration per assay and an assay temperature 5°C higher than in conditions used previously [10]. Suhayda [33] found higher florescence quench rates in plasma membrane isolated from seedlings of salt-stressed wild and cultivated barley species, but detected no change in ATP hydrolysis rate.

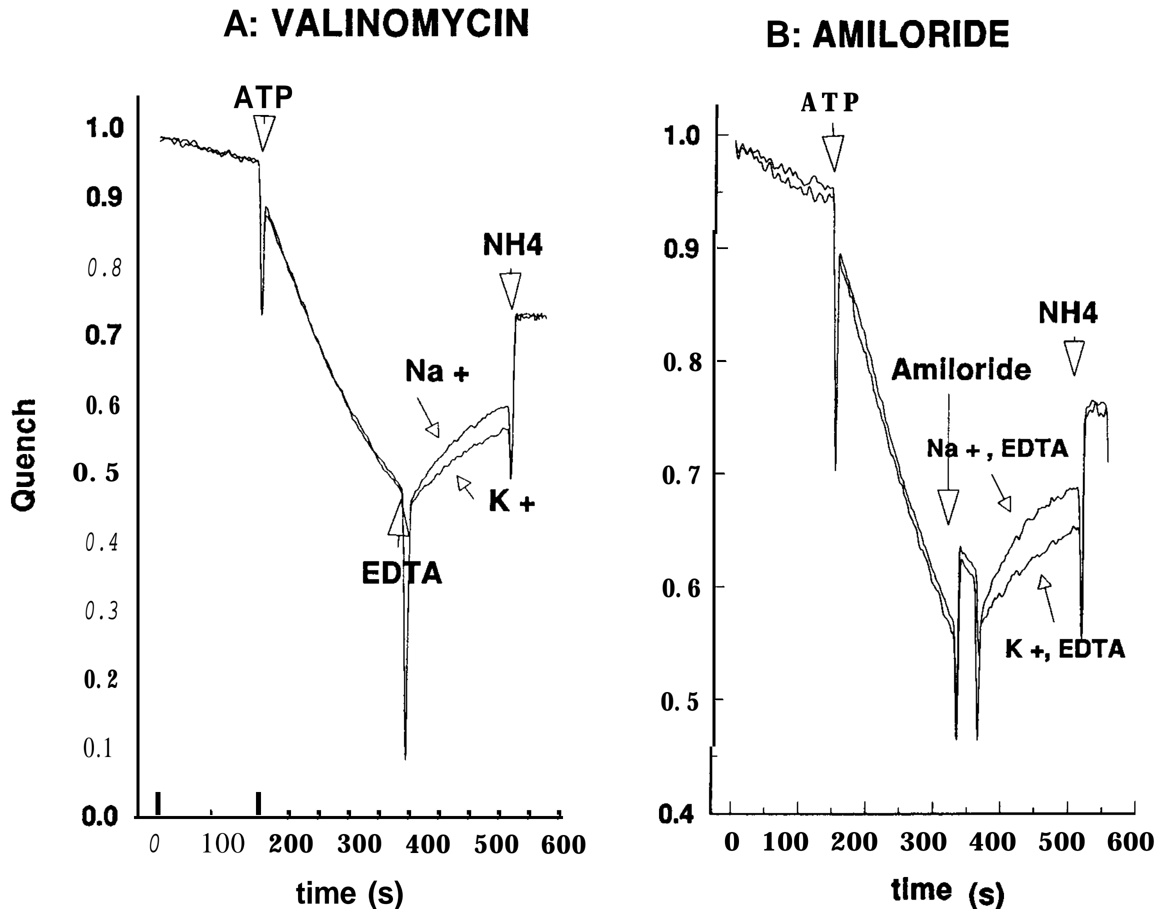

Fig. 2. Effect of 1 μM valinomycin and 0.5 mM amiloride on the rate of dissipation of the ATP-generated pH gradient in plasma membranes isolated from 50 mM salt-grown tomato roots (L. ***cheesmanii,*** ecotype 1401). Experimental procedures were as described in Fig. 1. Valinomycin or amiloride along with either 30 mM Na-gluconate or K-gluconate were added as indicated by the arrow.

Likewise, tonoplasts isolated from salt-treated barley seedlings exhibited increased proton transport rate without a concomitant increase in ATP hydrolysis [34]. The results reported herein clearly demonstrate an increase in both ATP hydrolysis rates and proton gradient formation in the plasma membrane of cultivated and wild tomato species isolated from roots grown under saline conditions.

Tomato, like other species, maintains growth under saline conditions by minimizing the concentrations of salt at active metabolic sites. This may involve the extrusion of Na+ ions from the cytosol via export across the plasmalemma and import into the vacuole. Previous studies with cultivated and wild tomato species indicate that Na+ is taken up more readily by the wild species, but that the cultivated species restricts uptake of this ion more effectively from both the root and shoot [11,35]. In an earlier work, Mennen et al. [24] reported that a Na+/H+ antiport mechanism may be operating in both the plasmalemma and tonoplast of *L. esculentum.* This would help to explain, in part, the exclusion of Na+ from the root and shoots of the cultivated tomato. However, in the present study using isolated membrane vesicles, there was no clear evidence of a Na+/H+ antiport mechanism in the tonoplast of either the cultivated or wild tomato species. Salt treat-

ment did not change this response. The conflict between the results of Mennen et al. [24] and ours with respect to the tonoplast may be explained by the fact that they used a different cultivar of tomato ('Holit') than used in this study ('Heinz 1350') and used different techniques involving the generation of an artificial pH gradient and assessing Na+ fluxes conducted in whole tissues. Variations in salt tolerance within *L. esculentum* have been reported [36,37]. Another possibility for the noted differences is that the antiport mechanism is extremely labile and was lost during the membrane isolation process. We included two protease inhibitors, PMSF and chymostatin, in our isolation; however, it still remains possible that the antiport mechanism was damaged. Alternatively, some regulatory protein needed for proper functioning could have been removed.

There was also no evidence for an antiport mechanism in the plasmalemma of either tomato species when the plants were grown under nonsaline conditions. However, an increase in Na+-stimulated recovery of fluorescence was demonstrated in plasma membranes isolated from salt-grown roots. This increase was evident even in the presence of 1 μM valinomycin plus K-gluconate indicating that this observation cannot be attributed to passive, electrochemically coupled cation/H+ exchange. Additionally, if Na+ were diffusing down its chemical gradient, it would be reasonable to expect to see some evidence of this in the control membranes also. However, there was no difference between the addition of K+ gluconate and Na+ gluconate to plasma membrane vesicles isolated from nonsalinized, control plants (Fig. 1A).

Furthermore, the inclusion of molybdate (1 mM), azide (1 mM) and nitrate (50 mM) in the assay media did not reduce the intensity of antiport activity in vesicles isolated from salt-stressed roots (data not shown). This would support the contention that antiport activity was not due to contamination by golgi, mitochondria, or tonoplast vesicles. Thus, at present the difference between the rates of fluorescence recovery in the presence of Na+ and K+ can best be explained by the operation of a Na+/H+ antiport mechanism. Consistent with this conclusion is the finding that the initial rate (Q_1) and near steady-state rate (Q_4) of pH gradient formation in the presence of NaCl was less than that obtained with KCl; and both NaCl and KC1 stimulated ATP hydrolysis similarly (Table 3) [9]. In membranes isolated from plants grown under saline conditions, the difference in fluorescence recovery with the addition of Na+ gluconate was highly significant as compared with the addition of K+ gluconate. The consistency of this response in plasma membranes isolated from different species and different cultivars (data not presented) and the absence of this response in plasma membranes grown in the absence of salinity was taken as clear evidence of a salinity-inducible Na+/H+ antiporter (Table 4).

Na+/H+ antiport activity in tomato root plasma membranes may not be ubiquitous. Antiport activity may be specific to vesicles of a particular root tissue and the low value of Na+/H+ antiport activity in salt-stressed plasma membrane could be due to its occurrence in specific cell types within the root and not in others. Ewing and Bennett [38] have recently reported the occurrence of six different P-type ATPases in tomato.

An interesting outcome of this study was the dramatic increase in ATP hydrolytic activity in plasma-membrane vesicles isolated from roots obtained from plants grown at salinity levels of 50 mM and 100 mM as well as increased proton pumping activity. Previously, we have not reported such large increases [9,10]. One possibility which might account for the differences is the inclusion of 5 $\mu g \cdot ml^{-1}$ chymostatin, a potent protease inhibitor, in our microsomal pellet suspension buffer. Also, unpublished results from our laboratory indicates that the increase in ATP hydrolytic activity is dependent on the salt concentration in the growth media such that earlier studies may have missed the critical salinity concentration.

In any case, these data are consistent with recent studies on NaCl regulation of plasma membrane H+-ATPase gene expression. Earlier Niu et al. [39] have reported that plasma membrane H+-ATPase mRNA accumulated in *Atriplex* and *Nicotiuna* roots after their exposure to salt (NaCl) treatments. Further work by the same group indicates that enhanced H+-transport activity induced by NaCl in *Atriplex* is mediated, at least in

part, by transcriptional or post-translational processes [40].

In contrast to animal tissue [30-32], sugar beet tonoplast vesicles [13], and *Dunaliella* plasma membrane [22], amiloride had little effect on the Na+/H+ exchange in plasma membrane vesicles isolated from salt-grown *L. cheesmanii.* This result is in agreement with results of Garbarino and DuPont [15] for tonoplast vesicles from salt-grown barley. Also, as in barley roots the Na+/H+ exchange was completely inducible by salt stress. Collectively, these findings suggest that there may be differences in the Na+/H+ exchange mechanism among plants. Efforts are now being made to detect antiport activity in interspecific Fl hybrids of the tomato species used in this study.

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