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REDUCTION IN PLASMA MEMBRANE ATPase ACTIVITY OF TOMATO ROOTS BY SALT STRESS

JOHN W. GRONWALD[a], CHARLES G. SUHAYDA[b], MOSHE TAL[c] and MICHAEL C. SHANNON[b,*]

[a]*USDA/ARS, Plant Science Research Unit, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108,* [b]*U.S. Salinity Laboratory, 4500 Glenwood Drive, Riverside, CA 92501 (U.S.A.) and* [c]*Department of Botany, Ben-Gurion University of the Negev, Beer Sheva, 84120 (Israel)*

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ATPase activity was characterized in a plasma membrane-enriched fraction isolated from tomato (*Lycopersicon esculentum* Mill., cv. Heinz 1350) roots grown in the absence or presence of salinity stress (−4 bars, 60 mM NaCl plus 12 mM $CaCl_2$). The enzyme exhibited the following properties in both non-stressed and salt-stressed roots: (1) activated by divalent cations ($Mg^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+} > Ca^{2+} > Zn^{2+}$) and further stimulated by monovalent cations ($Na^+ = K^+ > Rb^+ > Li^+$); (2) pH optima for Mg^{2+} activation and KCl-stimulation of 7.0 and 6.5, respectively; (3) selective for Mg^{2+}-ATP as substrate; (4) sensitive to *N,N'*-dicyclohexylcarbodiimide and vanadate but insensitive to azide and oligomycin; (5) not stimulated synergistically by Na^+ plus K^+. Exposing roots to salt-stress altered the kinetics of Mg^{2+}-ATPase activity. Simple Michaelis-Menten kinetics were observed when Mg^{2+}-ATP was used as substrate for both control and salt-treated roots. Salt-stress had little effect on the apparent K_m for Mg^{2+}-ATP. The predominant effect of salt-stress was to reduce V_{max} of Mg^{2+}-ATPase activity from 69 μmol P_i (mg protein)$^{-1}$ h^{-1} in control roots to 39 μmol P_i (mg protein)$^{-1}$ h^{-1} in salt-treated roots

Key words: salinity; salt-stress; plasma membrane; ATPase

Introduction

As a result of the increased demand for food crops and plant products, the use of irrigated agriculture in the world has increased approximately 300% during the past 35 years [1]. The rapid expansion in irrigation combined with increases in the use of saline irrigation waters has led to decreases in crop productivity primarily due to salinity stress [1]. The breeding and development of salt-tolerant plant cultivars represents a potential solution to this problem. However, in order to effectively select and breed for salt tolerance, physiological and/or biochemical traits that confer salt tolerance must be identified.

An important component of salt tolerance is the ability to regulate ion accumulation at the soil/root interface. An enzyme considered to play a major role in regulating ion transport at this interface is the root plasma membrane ATPase. It is well established that this enzyme functions as an electrogenic H^+-pump which acts as the primary active transport mechanism driving numerous secondary transport mechanisms [2]. This enzyme, which is activated by Mg^{2+} and further stimulated by K^+, is referred to as a Mg^{2+}-dependent ATPase (Mg^{2+}-

*To whom requests for reprints should be addressed.

Abbreviations: ANSA, 1-amino-2-naphthol-4-sulfonic acid; BSA, bovine serum albumin; DCCD, *N,N*-dicyclohexylcarbodiimide; DES, diethylstilbestrol, DTT, dithiothreitol; EDTA, ethylenediamine tetraacetic acid; EGTA, ethylene glycol-bis (β-aminoethyl)-*N,N'*-tetraacetic acid; FCCP, carbonyl cyanide *p*-trifluromethoxyphenylhydrazone; MES, 2-(*N*-morpholino)ethanesulphonic acid; P_i, inorganic phosphate; PNPP, *p*-nitrophenyl phosphate; UDPase, uridine diphosphatase.

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ATPase) [3] or as a proton translocating ATPase (H^+-ATPase) [2].

Studies concerning the effect of salinity stress on the plasma membrane H^+-ATPase of either glycophytic or halophytic species are limited. Braun et al. [4] reported that growing *Atriplex nummularia*, a halophyte, in hydroponic culture containing 400 mM NaCl enhanced H^+-translocating, Mg^{2+}-ATPase activity of sealed microsomal vesicles isolated from roots. Erdei et al. [3] examined Mg^{2+}-ATPase activity of crude microsomal fractions isolated from roots of *Plantago maritima* (a salt-tolerant species) and *P. coronopus* (a moderately salt-sensitive species) grown in the presence of salt. Mg^{2+}-ATPase activity isolated from roots of *P. maritima* decreased when NaCl levels in the hydroponic culture exceeded 150 mM. In the case of *P. coronopus*, Mg^{2+}-ATPase activity was reduced approximately 30 and 45% when plants were hydroponically-cultured in a medium containing 75 and 150 mM NaCl, respectively. H^+-ATPase activity of sealed microsomal vesicles isolated from roots of cotton, a moderately salt-tolerant glycophyte, was not altered by hydroponic culture in a medium containing 75 mM NaCl [5]. Douglas and Walker [6] examined the effect of salt-stress on vanadate-sensitive, Mg^{2+}-ATPase of a membrane fraction isolated from roots of citrus, a salt-sensitive glycophyte. The effect of salt exposure on Mg^{2+}-ATPase activity was genotype-dependent. Salt-exposure (50–100 mM NaCl) had no effect on Mg^{2+}-ATPase activity of two genotypes but resulted in increased activity of another genotype. Crude microsomal fractions were used in most of the above studies [3–5], hence whether the observed effects were specific for plasma membrane-ATPase is uncertain.

The domestic tomato (*Lycopersicon esculentum*) is a glycophytic species exhibiting a moderate degree of salt tolerance [7,8]. The purpose of the present study was to characterize the plasma membrane H^+-ATPase of tomato roots and to determine the effects of salt-stress on the properties of this enzyme. Our results indicate that tomato root plasma membrane H^+-ATPase exhibits substrate specificity, pH optima, cation requirements, and kinetics similar to plasma membrane ATPases isolated from other plant sources. The predominant effect of salinity stress is to decrease the V_{max} of H^+-ATPase activity.

Methods

Plant culture

Seeds of tomato (*Lycopersicon esculentum* Mill. cv. Heinz 1350) were treated for 15 min with 2.6% (w/v) NaOCl, rinsed for 30 min in deionized wate and placed on filter paper saturated with 0.1 mM $CaCl_2$. Seeds were germinated for 9 days in the dark at 25°C. Seedlings were then transferred t aerated hydroponic culture (half-strength Hoagland' nutrient solution, pH 6.0) in a growth chamber. A 16-h/day photoperiod was provided by fluorescen and incandescent bulbs to give photosynthetically active radiation of 240 μE m^{-2} s^{-1}. Day/night tem peratures were 30/25°C. After 2 weeks of growth seedlings were cultured hydroponically in either the growth chamber or greenhouse. The plants were grown in black plastic buckets (4 plants/bucket) con taining 15 l of aerated nutrient solution. The nutrien solution contained 2.5 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM KH_2PO_4, 0.1 mM Fe as chelated sodium ethylenediamine tetra acetate, 23 μM H_3BO_3, 4.5 μM $MnCl_2$, 0.4 μM $ZnSO_4$, 0.15 μM $CuSO_4$, and 0.05 μM H_2MoO_2 Three days after transfer, salt was added at the rate of 1 bar/day (15 mM NaCl plus 3 mM $CaCl_2$) for 4 days. The electrical conductivity of the control solu tion was approximately 1.2 dS (deciSeimens) m^{-1} and that of the plus salt-treatment was approxi mately 10.0 dS m^{-1}. The pH of the nutrient solu tions were maintained between pH 6.0 and pH 7.0 by periodic adjustment with H_2SO_4. Nutrient solu tions were changed weekly after the first 2 weeks Plants were 40–50 days old at harvest. Exposure to −4 bars of salinity reduced root and shoot growth of the tomatoes by 21 and 43% respectively. Plants grown in the growth chamber were used to charac terize the distribution of marker enzymes in mem brane fractions (Tables I and II). All other data were obtained from plants grown in the greenhouse with a normal unsupplemented photoperiod.

Plasma membrane isolation

A plasma membrane-enriched fraction was iso lated from roots of tomato using the procedure o Leonard and Hotchkiss [9] with minor modifications All operations were performed at 0–4°C. Roots (35 g) were excised, rinsed with cold, deionized wate

and ground with a mortar and pestle for 3 min in 150 ml of grinding medium which contained 0.25 M sucrose, 3 mM EDTA, 2.5 mM DTT and 25 mM Tris–MES (pH 7.9). The final pH after grinding was 7.3. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at $13000 \times g$ for 15 min. The supernatant fraction was then centrifuged for 30 min at $85000 \times g$ in a Beckman 45-Ti rotor to collect the microsomal fraction. The microsomal pellet was resuspended in 4 ml of suspension medium containing 1 mM Tris–MES and 1 mM DTT at pH 7.2 and the resulting suspension was layered onto two 36-ml discontinuous gradients (2 ml/gradient) consisting of 28 ml of 45% (w/w) and 8 ml of 34% (w/w) sucrose containing 1 mM DTT and 1 mM Tris-MES (pH 7.2). The gradient was centrifuged for 2 h at $82000 \times g$ in a Beckman SW-28 rotor. The plasma membrane fraction was collected from the 34–45% sucrose interface and stored in liquid nitrogen for up to 1 week before assay. Storage for this period of time had no effect on enzyme activity.

Enzyme assays

The standard assay for ATPase activity was carried out at 38°C in a 1.0 ml volume containing 3 mM ATP (Tris-salt), 3 mM $MgSO_4$, 30 mM Tris–MES (pH 6.5) and 50 mM KCl when added. The exact composition of the assay medium is provided in the relevant tables or figure legends. The reaction was initiated by the addition of 10–20 μg of membrane protein. After 30 min (15 min in the case of the kinetic studies with Mg^{2+}-ATP), the reaction was stopped by the addition of 0.8 ml of a 10% (w/v) sodium dodecyl sulfate (SDS) solution. The amount of inorganic phosphate released was determined by the method of Peterson [10] except that ANSA was used at a concentration of 0.05% (w/v). Under our assay conditions, ATPase activity was linear for at least 45 min. Latent UDPase activity was measured as described by Nagahashi and Nagahashi [11]. Cytochrome *c* oxidase was assayed as described by Dupont et al. [12] and antimycin A insensitive NADH-cytochrome *c* reductase as described by Leonard and Hodges [13]. Kinetic constants were obtained from Lineweaver-Burk plots using the FORTRAN program of Cleland [14].

Protein determination

Protein was estimated by the procedure of Peterson et al. [15] using BSA as a standard.

Chemicals

Triton X-100 was obtained from Calbiochem, SDS (low phosphate) from Matheson, Coleman & Bell and ANSA from Kodak. All other reagents were obtained from Sigma.

Results

Distribution of marker enzymes

The protein concentrations and activities of various marker enzymes associated with fractions collected by differential and sucrose density gradient centrifugation during the isolation of plasma membrane-enriched microsomes are shown in Tables I and II. The total amount of protein measured in the crude homogenate of salt-stressed roots was generally less than that measured from control roots (Table I). The reason for this is not clear. However, in subsequent fractions obtained during isolation, the distribution of protein and marker enzymes was similar in control and salt-treated roots. Because the distribution was similar, the data for the marker enzymes NADH cytochrome *c* reductase, cytochrome *c* oxidase and latent UDPase are shown only for control membranes (Table II).

The membrane fraction collected at the 34/45% sucrose interface contained approximately 0.6% of the total protein of the root homogenate and was enriched in plasma membranes as indicated by the high level of vanadate-sensitive ATPase activity (Tables I and II). Contamination by endoplasmic reticulum, Golgi, and mitochondria was minor, as indicated by the marker enzymes NADH cytochrome *c* reductase, cytochrome *c* oxidase and latent UDPase, respectively (Table II).

Linear sucrose gradient (10–45%, w/w) analysis of microsomal fractions from control and salt-stressed tomato roots showed no significant shifts in sedimentation density of essential marker enzymes as a result of salt-exposure. The following sucrose densities were found for peak enzyme activities from control and salt-stressed membrane fractions respectively: Triton X-100 stimulated UDPase (34.8%, 33.3%), KCl-stimulated, Mg^{2+}-dependent ATPase (36.0%, 36.0%), cytochrome *c*

Table I. Distribution of protein and ATPase activity in fractions isolated from roots of control and salt-stressed tomato plants

Fraction	Total protein[a] (mg)		ATPase specific activity[b] (μmol P_i (mg protein)$^{-1}$ h^{-1})	
	Control	Salt	Control	Salt
Root homogenate	230 (37)[c]	180 (30)	49 (6)	49 (8)
13000 × *g* centrifugation				
Pellet	35 (9)	30 (6)	36 (8)	41 (9)
Supernatant	82 (11)	77 (8)	60 (12)	49 (9)
85000 × *g* centrifugation				
Pellet	12 (3)	10 (4)	55 (11)	32 (11)
Supernatant	65 (12)	46 (12)	42 (9)	27 (8)
Sucrose gradient				
Non-migrating band	2.0 (1.2)	2.8 (1.0)	24 (8)	21 (7)
34%/45% interface	1.8 (0.6)	1.3 (0.5)	56 (8)	35 (6)
45% pellet	0.4 (0 4)	0 6 (0.5)	–[d]	–

[a] Based on 100 g fresh weight.
[b] Assay mixture contained 3 mM $MgSO_4$, 3 mM ATP and 30 mM Tris–MES (pH 6.5)
[c] Numbers in parentheses are standard deviations based on 3 extractions.
[d] Not determined.

Table II. Distribution of vanadate-sensitive ATPase activity and marker enyzmes in membrane fractions isolated from tomato roots.

Fraction	Vanadate-sensitive ATPase activity[a] (%)	Specific Activity		
		Latent UDPase[b]	NADH Cyt *c* reductase[c]	Cyt *c* oxidase[c]
13000 × *g* centrifugation				
Pellet	57	3 0 (22)[d]	7 7 (21)	20.8 (90)
Supernatant	15	3 6 (78)	9.8 (79)	0 8 (10)
85000 × *g* centrifugation				
Pellet	51	19.0 (47)	25 4 (23)	4.9 (7)
Supernatant	–[e]	–	8 8 (59)	0.7 (8)
Sucrose gradient				
Non-migrating band	45	24.6 (10)	14 3 (2)	4 4 (1)
34%/45% interface	57	3 4 (1)	11.3 (1)	9 6 (2)
45% pellet	32	1.8 (< 1)	4 4 (< 1)	4.7 (< 1)

[a] Assay mixture contained 3 mM $MgSO_4$, 3 mM ATP, 30 mM Tris–MES (pH 6 5), 50 mM KCl and 50 μM Na_3VO_4, results expressed as percent of activity without Na_3VO_4
[b] Assay mixture contained 3 mM $MnSO_4$, 3 mM UDP, and 30 mM Tris–MES (pH 6.5) Triton X-100 stimulated activity expressed in μmol P_i (mg protein)$^{-1}$ h^{-1}
[c] Activity expressed as μmol (mg protein)$^{-1}$ h^{-1}
[d] Numbers in parentheses indicate activity as a percent of total activity in the combined 13000 × *g* fraction.
[e] Not determined

Table III. Influence of divalent cations on ATPase activity and the nucleotide specificity of phosphatase activity of plasma membrane fractions from tomato roots grown with or without salt.

	ATPase activity (μmol P_i (mg protein)$^{-1}$ h^{-1})			Phosphatase activity (μmol P_i (mg protein)$^{-1}$ h^{-1})	
Cation[a] added	Control	Salt-treated	Substrate added	Control	Salt-treated
			ATP	52.5	45.5
None	13.0	3.6	ADP	3.9	1.9
$MgSO_4$	71.2	41.9	CTP	2.8	0.1
$MnSO_4$	56.8	34.8	UTP	5.0	2.3
$CoSO_4$	37.6	21.2	UDP	5.8	3.1
$NiSO_4$	24.1	13.3	GTP	8.9	4.8
$CaSO_4$	18.0	4.5	ITP	8.4	4.9
$ZnSO_4$	12.7	2.9	IDP	5.2	2.3
			PNPP(pH 5.0)	5.4	1.7
			PNPP(pH 6.0)	2.3	0.1

[a] Assay mixture contained 3 mM ATP, 30 mM Tris–MES (pH 6.5 except as indicated) and when added, 3 mM divalent cation.
[b] Assayed in the presence of 3 mM substrate (sodium salt), 3 mM $MgSO_4$ and 30 mM Tris–MES (pH 6.5).

oxidase (39.4%, 39.7%). For routine isolations of plasma membranes, a 34/45% discontinuous gradient resulted in a high yield of plasma membrane with minimum contamination from other membranes.

Characterization of plasma membrane ATPase activity

The ATPase activity associated with the plasma membrane-enriched fraction isolated from cv. Heinz tomato roots exhibited properties similar to those previously reported for plasma membrane ATPase isolated from another tomato cultivar [16] and other plant species [9,12,13,17,18]. Exposure of plants to −4 bars of salt (60 mM NaCl plus 12 mM $CaCl_2$) had little or no effect on divalent cation activation (Table III), monovalent cation stimulation (data not shown), pH optimum (Fig. 1), substrate specificity (Table III) and inhibitor sensitivity of membrane preparations (Table IV).

ATPase activity was divalent cation-dependent with Mg^{2+} being the most effective divalent cation in activating ATPase activity (Table III). The addition of monovalent cations further stimulated Mg^{2+}-dependent ATPase activity. The alkali metals K^+, Na^+, and Rb^+ provided equivalent stimulation and Li^+ provided the least (data not shown). The possible synergistic stimulation of Mg^{2+}-ATPase activity by a combination of K^+ and Na^+ ions was examined by maintaining the total monovalent cation

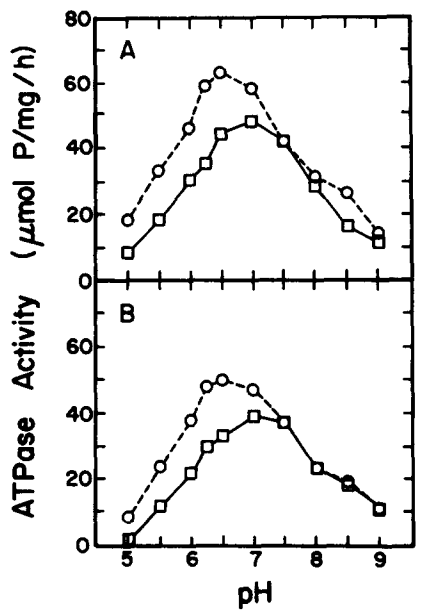

Fig. 1. Effect of pH on ATPase specific activity, measured in the absence (□) and presence (○) of KCl, of a plasma membrane fraction isolated from control (A) and salt-stressed (B) roots. Assay mixture contained 3 mM $MgSO_4$, 3 mM ATP, 30 mM Tris–MES (pH range 5.0–8.5), 30 mM glycine-NaOH (pH 9.0) and 50 mM KCl (as indicated).

Table IV. Effect of inhibitors on ATPase activity of plasma membranes isolated from tomato roots grown in the presence or absence of salt.

Treatment[a]	ATPase activity (% of control)	
	Control	Salt-treated
Control	100 (46.8)[b]	100 (28.8)
Oligomycin (5 μg/ml)	94	92
NaN_3(1 mM)	85	86
DCCD(50 μM)	32	28
DES(50 μM)	37	42
Na_3VO_4(500 μM)	33	25
Na_2MoO_4(1 mM)	95	98

[a] Assayed in the presence of 3 mM $MgSO_4$, 3 mM ATP and 30 mM Tris–MES (pH 6.5)

[b] 100% values in μmol P_i (mg protein)$^{-1}$ h^{-1}.

concentration at 50 mM while varying the Na^+:K^+ ratio. The results indicate that K^+/Na^+ synergism is not a property of the plasma membrane ATPase in either control or salt-treated roots (data not shown).

Tomato root plasma membrane ATPase had a pH optimum of 7.0 in the presence of Mg^{2+}-ATP (Fig. 1). The addition of 50 mM KCl shifted the optimum to 6.5. Similar shifts in pH optima upon K^+ addition have been reported for plasma membrane ATPase from oats (*Avena sativa*) [13], corn (*Zea mays*) [9] and red beet (*Beta vulgaris*) [17]. Possible explanations for these pH shifts have been postulated [19].

Enzyme activity in the plasma membrane-enriched fraction isolated from both control and salt-treated roots exhibited a strong preference for ATP as substrate (Table III). Activity measured in the presence of ATP was considerably greater than that measured in the presence of other di- and triphosphates tested. Potassium stimulation of enzyme activity was ATP-dependent.

ATPase activity was insensitive to inhibitors of mitochondrial ATPase (NaN_3, oligomycin) but was sensitive to known inhibitors of plasma membrane ATPase (DCCD, vanadate, DES) (Table IV). Nitrate (50 mM), which is known to inhibit tonoplast ATPase [2], caused only a 7% inhibition of ATPase activity (data not shown) indicating little contamination by tonoplast ATPase. Molybdate, an inhibitor of acid phosphatase [18] was ineffective in inhibiting ATPase activity. On the basis of the inhibitor effects, it is apparent that the plasma membrane fraction utilized in these studies was highly enriched plasma membranes. Exposure to salinity had no e fect on the inhibitor sensitivity of the plasma mer brane-enriched fraction (Table IV) indicating that tl purity of the plasma membrane fraction isolated fro salt-treated roots was comparable to that of contr roots.

Effect of salt-stress on the kinetics of ATPase activi

Simple Michaelis-Menten kinetics were found f ATPase activity isolated from both control and sal treated roots (Fig. 2). Values for the kinetic pa

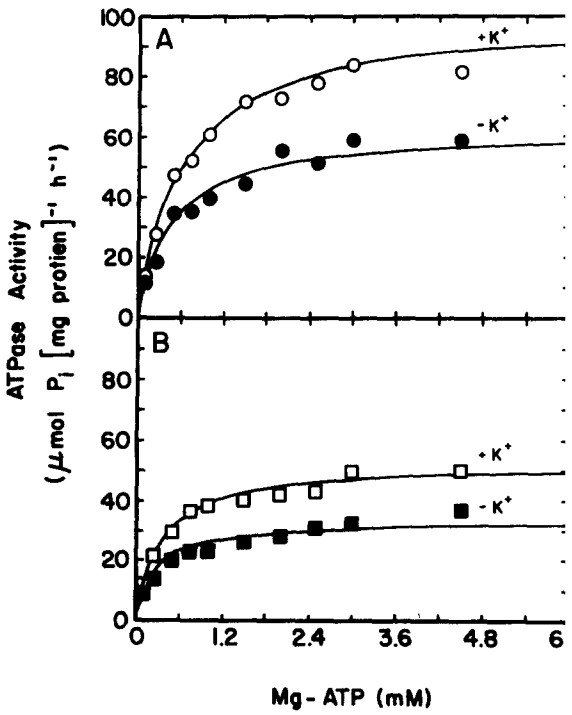

Fig. 2. Michaelis-Menten curves of plasma membrane ATPas specific activity in control (A) and salt-treated (B) roots as function of increasing MgATP concentration in the presence c absence of 50 mM KCl. Enzyme activity was measured at pl 6.5 in a medium containing 30 mM Tris–MES, 50 mM KCl (whe added) and equal concentrations of ATP and Mg^{2+} as indicatec Kinetic constants were obtained from Lineweaver-Burk plot [14]. (A) For control roots, the V_{max} and K_m were 69 μmol I (mg protein)$^{-1}$ h^{-1} and 0.64 mM, respectively in the absence c K^+ and 93 μmol P_i (mg protein)$^{-1}$ h^{-1} and 0.52 mM, respectivel in the presence of K^+. (B) For salt-treated roots, the V_{max} an K_m were 39 μmol P_i (mg protein)$^{-1}$ h^{-1} and 0.52 mM, respec tively in the absence of K^+ and 53 μmol P_i (mg protein)$^{-1}$ h$^-$ and 0.37 mM, respectively in the presence of K^+.

ameters, apparent K_m and V_{max}, were obtained from Lineweaver-Burk plots using the FORTRAN program of Cleland [14]. ATPase activity associated with control roots had V_{max} values of 69 and 93 μmol P_i (mg protein)$^{-1}$ h^{-1} when assayed in the absence and presence of 50 mM KCl, respectively. The apparent K_m value for Mg^{2+}-ATP decreased from 0.64 to 0.52 mM upon addition of 50 mM KCl. V_{max} values for Mg^{2+}-ATPase and KCl-stimulated, Mg^{2+}-ATPase are somewhat higher than those reported for plasma membrane-enriched fractions isolated from oat roots [13] and noticeably higher than those reported for corn [9] and red beet [17]. Anthon and Spanswick [16] reported that as compared to other plant sources of plasma membrane ATPase, tomato roots contain more plasma membrane ATPase per gram fresh weight.

Plasma membranes isolated from salt-stressed roots showed a substantial reduction in ATPase activity (Fig. 2B). Kinetic studies revealed that the primary effect of salt stress was to reduce V_{max}. V_{max} values for Mg^{2+}-ATPase and KCl-stimulated, Mg^{2+}-ATPase were 39 and 53 μmol P_i (mg protein)$^{-1}$ h^{-1}, respectively in salt-stressed roots. This represents a salt-induced reduction of the V_{max} of ATPase specific activity of about 43% when measured in the presence or absence of KCl in the assay media. Standard deviations of the means for V_{max} values were found to be only 2 and 3 μmol P_i (mg protein)$^{-1}$ h^{-1} for salt-stressed and control roots, respectively, for three repetitions in each of two different experiments. Although salt stress also reduced the apparent K_m values for Mg^{2+}-ATP in both the absence and presence of KCl, the magnitude of this decrease was less. In plasma membrane-enriched fractions isolated from salt-stressed roots, the apparent K_m value for Mg^{2+}-ATP decreased from 0.52 to 0.37 mM upon the addition of KCl.

Discussion

The protocol of Leonard and Hotchkiss [9], utilizing differential and density gradient centrifugation, yielded a plasma membrane-enriched fraction suitable for an initial characterization of the effects of salinity on the plasma membrane ATPase of tomato roots. The plasma membrane isolated from tomato roots displayed substrate specificity, ion requirements, pH optima and inhibitor sensitivity characteristic of plasma membrane ATPases isolated from other plant sources [2].

In contrast to what has been reported for corn [9] and oat [13] plasma membrane ATPase, monovalent cation stimulation of the enzyme isolated from tomato roots exhibited little selectivity. Among the monovalent cations, Na^+, K^+, Rb^+, there was little difference in ability to stimulate ATPase activity. In this respect, the plasma membrane of tomato roots was similar to that of red beet [17] and the halophyte, *Atriplex nummularia* [19]. Based on these limited comparisons, it appears that in salt-sensitive species such as corn and oats, the stimulation of ATPase activity by K^+ is much greater than that by Na^+, while in more salt-tolerant species (*Atriplex*, red beet, tomato) stimulation by these cations is equivalent.

Growing tomatoes hydroponically in nutrient solution containing 60 mM NaCl plus 12 mM $CaCl_2$ (−4 bars) had no effect on most characteristics of the plasma membrane ATPase (pH optima, inhibitor sensitivity, substrate specificity, effects of ions). However, exposure to this level of salinity did alter the kinetics of ATPase activity. Our results indicate that a major effect of salt-stress is the reduction of the specific activity of the ATPase. This effect is primarily due to the ability of salt-exposure to reduce V_{max}. A change in the apparent K_m of the enzyme for Mg^{2+}-ATP could not be statistically confirmed.

The reduction in V_{max} of ATPase activity as a result of salt-exposure is open to several interpretations. For instance, salt-stress may reduce the number of ATPase molecules/unit of plasma membrane surface area, perhaps through its effects on the rate of synthesis and/or degradation of the ATPase. Alternatively, salt-stress may not actually reduce the number of ATPase molecules per unit membrane surface area but may reduce the proportion of ATPase molecules to other membrane protein. This could occur if salt-stress increased the number of plasma membrane proteins, other than ATPase, resulting in a reduction in specific activity of ATPase. Lastly, it should be considered that salt-stress may impair the catalytic efficiency of the enzyme either by affecting the synthesis of positive or

negative effectors or through its effects on the lipid composition of the membrane.

Of the hypotheses discussed above, one that should receive attention in future research efforts is the modulation of ATPase activity as an indirect result of the effects of salt-stress on plasma membrane lipid composition. It is well-established that salt-stress can alter the lipid composition of membranes in plants [20]. In certain plant species, salt-stress has been reported to increase the free sterol content [20,21] or increase the sterol/phospholipid ratio [6] of microsomal membranes. These changes are considered to have adaptive significance because they decrease passive permeability to salt and increase membrane stability [20]. However, it is not certain what effect these changes have on an integral membrane protein such as the plasma membrane ATPase. There is considerable evidence, both in plant and mammalian systems, that modification of the lipid composition of a membrane can alter the kinetic properties of an integral membrane protein such as the plasma membrane ATPase [22,23]. For example, increased ordering of the membranes of red blood cells, as a result of an increase in sterol content or modification of fatty acyl composition, reduced the catalytic efficiency of the $(Na^+ + K^+)$-ATPase [24]. In plants, there are also indications that membrane lipid composition may affect the catalytic efficiency of the plasma membrane ATPase. Douglas and Walker [6] reported a correlation between the Arrhenius energy of activation (E_a) of Mg^{2+}-ATPase activity of a plasma membrane fraction isolated from citrus roots of 3 genotypes and the sterol/phospholipid ratio of the membrane fraction. A higher E_a for ATPase activity was observed in plasma membranes fractions which exhibited a higher sterol/phospholipid ratio. The authors [6] postulated that the increase in E_a was related to the ability of sterol to reduce membrane fluidity.

In the halophyte, *Plantago maritima*, salt exposure caused a decrease in ATPase activity measured in a plasma membrane-enriched fraction isolated from roots [25]. After 4 weeks of growing in a nutrient medium containing 200 mM NaCl, the V_{max} values for ATPase activity measured in the plasma membrane-enriched fractions isolated from the control and salt-treated roots were 33.5 and 11.0 μmol P_i (mg protein)$^{-1}$ h^{-1}, respectively. In contrast, the V_{max} of the plasma membrane ATPase solubilized (using 1% lysophosphytidylcholine) from salt-treated roots was greater than that from control roots (0.24 vs. 0.13 μmol P_i (mg protein)$^{-1}$ h^{-1} from salt-treated and control roots, respectively). These conflicting results may be due to differences in purity between the solubilized ATPase fractions and the plasma membrane-enriched microsomal fractions. Alternately, they may relate to the influence of the lipid environment on the catalytic properties of the enzyme. As discussed above, the lipid environment surrounding the enzyme may modulate activity and kinetic constants measured in lysophosphytidylcholine-solubilized vesicles may not reflect those in situ.

In summary, the results of this study have shown that salt-stress reduces the V_{max} of ATPase activity measured in plasma membrane-enriched fractions isolated from tomato roots. Additional study is needed to determine what effect this change has on in vivo ion transport capacity of tomato roots and whether this change in kinetic properties of the enzyme has adaptive significance or represents a deleterious effect of salt-stress.

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