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Na^+/H^+ and K^+/H^+ Antiport in Root Membrane Vesicles Isolated from the Halophyte *Atriplex* and the Glycophyte Cotton[1]

Miriam Hassidim, Yael Braun, Henri R. Lerner, and Leonora Reinhold*

Department of Botany, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel

ABSTRACT

Proton fluxes have been followed into and out of membrane vesicles isolated from the roots of the halophyte *Atriplex nummularia* and the glycophyte *Gossypium hirsutum*, with the aid of the ΔpH probe [^{14}C]methylamine. Evidence is presented for the operation of Na^+/H^+ and K^+/H^+ antiporters in the membranes of both plants. Cation supply after a pH gradient has been set up across the vesicle membrane (either as a result of providing ATP to the H^+-ATPase or by imposing an artificial pH gradient) brings about dissipation of the ΔpH, but does not depolarize the membrane potential as observed in similar experiments, but in the absence of Cl^-, using the ΔΨ probe SCN^-. Cation/H^+ exchange is thus indicated. This exchange is not due to nonspecific electric coupling, nor to competition for anionic adsorption sites on the membrane, nor to inhibition of the H^+-ATPase; coupling of the opposed cation and H^+ fluxes by a membrane component is the most likely explanation. Saturation kinetics have been observed for both Na^+/H^+ and K^+/H^+ antiport in *Atriplex*. Moreover, additive effects are obtained when Na^+ is supplied together with saturating concentrations of K^+, and vice versa, suggesting that separate antiporters for Na^+ and for K^+ may be operating. In the case of both *Atriplex* and *Gossypium* evidence was obtained suggesting the presence of antiporters in both plasmalemma and tonoplast.

In view of their obvious potential role in the adaptation of plants to high ambient salt concentrations, we have been studying membrane transport mechanisms in plants of varying resistance to salinity (6, 7, 10). We have previously reported on proton-translocating ATPase activity in membrane vesicles isolated from the roots of the halophyte *Atriplex nummularia* and the glycophyte cotton, a crop plant resistant to mild saline stress (6, 10). We detected and reported on Na^+/H^+ antiporter activity in *Atriplex* root membranes (7). This was also recently observed in membrane from *Atriplex* leaf membranes (13). This paper presents evidence suggesting that two antiporters may operate in *Atriplex*, one for Na^+ and one for K^+. Further, we report on our search for such antiporter mechanisms in the glycophyte cotton.

Na^+/H^+ antiport activity has been reported in two other higher plants as well as in *Atriplex*, both of them salt tolerant (1, 9), as well as in two algae (8, 12). K^+/H^+ antiport has been deduced in the case of two glycophytes (16, 17). In neither case was the effect specific for K^+, considerable activity being noted for other monovalent cations as well. In all the higher plants, apart from *Atriplex* roots (7) and zucchini hypocotyl (16), the antiporter activity described was present in the tonoplast. We have obtained evidence for the operation of antiporters in the plasmalemma as well as the tonoplast, both in cotton and in *Atriplex*. Some of these results have been briefly presented in a previous communication (5).

MATERIALS AND METHODS

Plant Material and Isolation of Vesicles

Atriplex nummularia and *Gossypium hirsutum* L. var Acala San Jose 2 were grown as described before (6, 10) in aerated half-strength Hoagland-Snyder solution (11), with or without the addition of 400 mM NaCl in the case of *Atriplex*. Root tips were sampled as before (6) and homogenized in a medium containing 0.25 M sucrose, 2 mM EGTA, 5 mM $MgSO_4$, 1% BSA, 25 mM BTP[2]-Mes (pH 7.2) with the addition of 100 mM ascorbic acid and 3% (w/v) PVP in the case of cotton. After centrifugation at 13,000*g* for 15 min the supernatant was recentrifuged at 100,000*g* (Beckman ultracentrifuge L5-50B, 50.2 Ti) for 30 min and the pellet resuspended with a syringe (25 gauge needle) in 2 mL suspension medium containing 1 mM $MgSO_4$, 1% BSA, 5 mM BTP-Mes (pH 7.2). This hypoosmotic shock causes leakage of internal vesicular solutes, thereby decreasing buffering capacity and improving ΔpH measurements without affecting ΔΨ measurements. The crude microsome preparation thus obtained was layered on a 20 to 45% (w/v) six-step discontinuous sucrose gradient (6) containing 1 mM $MgSO_4$, 1% BSA, 1 mM BTP-Mes (pH 7.2), and centrifuged at 80,000*g* for 2 h (SW 41). The membrane fractions collected at the 20/25%, 25/30%, and 30/34% interfaces (fractions 3, 4, and 5, *i.e.* those usually regarded as 'tonoplast enriched') were pooled and stored at −80°C. The membrane fractions collected at the 34/38% and 38/45% interfaces (fractions 6 and 7, *i.e.* those usually regarded as 'plasma membrane enriched') were also pooled and stored at −80°C. For the experiments the thawed membranes were diluted in 4 volumes of the suspension medium and pelleted at 100,000*g* for 30 min (SW 41). The pellet was resuspended in the suspension medium containing 0.25 M sorbitol, aiding dispersal with a syringe.

[1] Supported by a grant from the U.S.-Israel Binational Agricultural Research and Development fund (BARD).

[2] Abbreviation: BTP, bis-tris propane (1,3,bis[tris(hydroxymethyl)methylamine]propane.

Measurement of ΔpH

Gradients in pH across the vesicle membranes were measured by following the accumulation of ^{14}C-labeled CH_3NH_2. Aliquots of 50 μL of the resuspended membrane preparation (containing 30–80 μg protein) were added to 10 μL medium containing 67 mM sucrose, 5 mM $MgSO_4$, 1% BSA, 30 μM $^{14}CH_3NH_2$ (1.1×10^4 Bq/μmol final specific activity), 1.5 mM BTP-Mes (pH 7.0), with or without 5 mM Tris-ATP (final concentrations); with the addition of 20 mM BTP-Cl or 40 mM choline chloride in the case of cotton or 100 mM BTP-Cl in that of *Atriplex*. These chloride concentrations depolarize the membrane potential. The experiments were carried out at 20°C. The reaction was terminated by dilution in 2 mL of a solution containing 0.25 M sucrose, 3 mM $MgSO_4$ (pH 7.0) and the vesicles were then rapidly filtered on Millipore HAWP filters, pore size 0.45 μm, and rinsed twice with 2 mL of the same solution. The procedure was completed in 15 s. The filters were then transferred to scintillation vials containing toluene-Triton scintillation fluid and counted in a scintillation counter (Beckman LS 1801).

'pH Jump' Experiments

Vesicles were loaded by a hypoosmotic shock with suspension medium buffered to pH 5.5. An artificial pH gradient was imposed across the vesicle membranes by diluting them in buffer solution pH 7.0. The thawed membranes were diluted in 4 volumes of suspension medium (pH 5.5), and centrifuged at 100,000*g* for 30 min. The pellet was resuspended in one volume of suspension medium (pH 5.5) containing 0.25 M sorbitol, to provide a final vesicle concentration about twice that employed in the H^+-ATPase experiments. Aliquots of 20 μL containing the same amount of protein as in the H^+-ATPase experiments, *i.e.* 30–80 μg, were added to 40 μL medium containing 0.25 M sorbitol, 5 mM $MgSO_4$, 1% BSA, 30 μm $^{14}CH_3NH_2$ (1.1×10^4 Bq/μmol final specific activity), 50 mM BTP-Mops (pH 7.0), and, in the case of cotton vesicles, 20 mM BTP-Cl or 40 mM choline chloride; in the case of *Atriplex* vesicles 40 mM BTP-Cl. The experiments were carried out at 10°C. The reaction was terminated by dilution as described previously.

Protein Determination

Protein was determined in the membrane fractions by the method of Bradford (4).

Chemicals

$^{14}CH_3NH_2$ was obtained from the Radiochemical Centre Amersham, U.K. Na_3ATP was supplied by Boehringer Mannheim, and was passed through a Tris-Dowex 50 column to yield Tris-ATP.

RESULTS

The Na^+/H^+ antiporter which we previously reported in *Atriplex* (7) was detected in membrane fractions 6 + 7 (the plasmalemma-enriched fraction). The data in Figure 1A suggests that the plasma membrane-enriched fraction from cotton also possesses cation/H^+ antiport activity. After a ΔpH was established by supplying MgATP to the H^+-ATPase present in these membrane vesicles (10) subsequent addition of KMes or NaMes brought about partial dissipation of the ΔpH (Fig. 1A). In contrast to the situation in *Atriplex* (Fig. 1B) K^+ was the more effective cation. The cation concentrations tested were lower in the case of cotton because the threshold for inhibition of the H^+-ATPase was lower for cotton than for *Atriplex*. Similar data were also obtained for membrane fraction 3 + 4 + 5 indicating the presence of cation/H^+ antiport activity in the tonoplast (not shown).

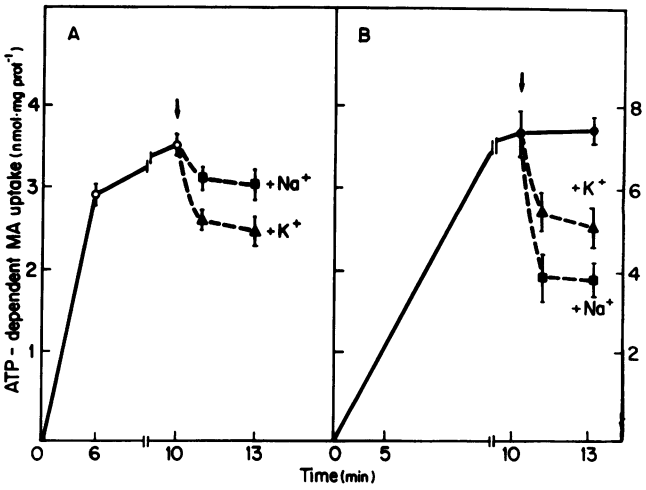

Figure 1. Effect of adding NaMes (■) or KMes (▲) on the level of ATP-dependent methylamine (MA) accumulation in root membrane vesicles (fractions 6 and 7) isolated from cotton (A) or salt-grown *Atriplex* (B). ATP (5 mM) was supplied at time 0; the arrow indicates supply of the cations to a final concentration of 20 mM (A) or 100 mM (B). The medium (for composition see "Materials and Methods") contained 40 mM choline chloride (A) or 100 mM BTP chloride (B). The points give means of quadruplicates ± SE.

Before it can be concluded that the results shown in Figure 1 indicate cation/H^+ exchange mediated by a membrane component, several alternative explanations must be excluded.

(a) The opposed fluxes might be electrically coupled in consequence, for instance, of the generation of a diffusion potential.

(b) The protonated, positively charged form of the ΔpH probe might be adsorbed to anionic sites on the membrane such as anionic lipids (see ref. 14). Addition of K^+ or Na^+ might displace the probe from such sites.

(c) Addition of the cations might depress the activity of the H^+-ATPase.

To minimize the first possibility, all experiments were carried out in the presence of Cl^- in concentrations which prevented the formation of any $\Delta\Psi$ (see ref. 6 for data for *Atriplex*). In the case of cotton, 20 mM Cl^- was observed to depolarize the membrane almost completely and no potential was detectable in the presence of 40 mM Cl^-.

With regard to the second possibility, far less probe was displaced by the addition of 100 mM Na^+ or 5 mM K^+ in the absence of ATP than in its presence (see Fig. 2 for cotton

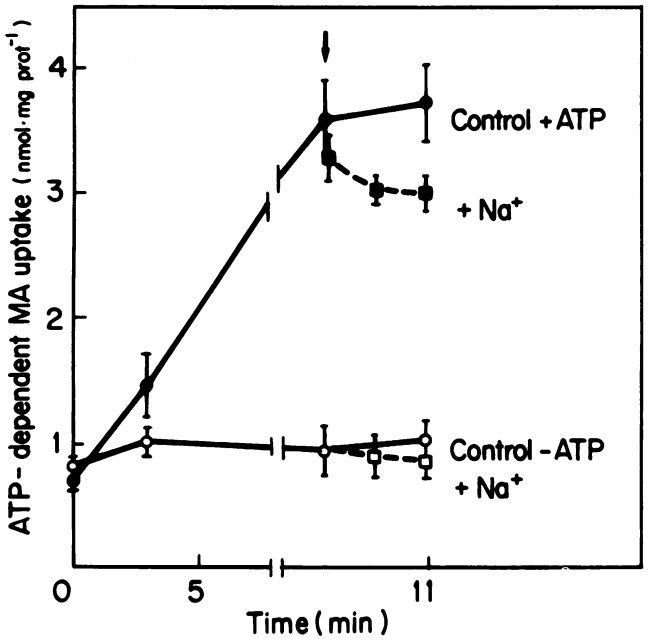

Figure 2. Effect of addition of NaMes on level of methylamine (MA) accumulation in cotton root membrane vesicles (fractions 6 and 7) in presence or absence of 5 mM ATP. NaMes was added at the arrow to a concentration of 100 mM. Medium as given in "Materials and Methods," BTP-Cl concentration 20 mM. Points give means of quadruplicates ± SE.

fraction 6 + 7, similar data were obtained for cotton fraction 3 + 4 + 5—not shown; for *Atriplex* see ref. 7). Anionic lipid sites would not be expected to increase as a result of ATP supply. Further, Figure 3 shows a pH jump experiment where an artificial ΔpH was imposed across the vesicle membrane by loading cotton vesicles, fraction 6 + 7, with buffer at pH 5.5 and diluting them into buffer at pH 7.0. Control vesicles were both loaded with, and diluted into, buffer at pH 7.0. Addition of K^+ in the latter case did not produce appreciable methylamine displacement, demonstrating that adsorption to anionic sites on the membrane surface is not involved in the 'antiport' effect.

With regard to the possibility that the cations might have depressed H^+-ATPase activity, the cation concentrations supplied in the experiments shown in Figure 1, A and B, did not inhibit the H^+-ATPase and, in fact, tend to stimulate it (7, 10).

Further, when H^+-ATPase activity was inhibited by the addition of EDTA after establishment of the ΔpH (Fig. 4), H^+/K^+ exchange was still exhibited. Addition of NaCl or LiCl also dissipated the ΔpH, but in the latter case to a considerably smaller extent. In Figure 4, dissipation was measured 30 s after supply of EDTA and cations, since longer incubation periods in the presence of EDTA alone resulted in proton leakage (not shown). Figure 4 shows data for cotton fraction 6 + 7, similar results were obtained for cotton fraction 3 + 4 + 5 (data not shown).

Are There Specific Antiporters for Na^+ and for K^+?

If only one H^+/monovalent cation antiporter were operating in the *Atriplex* vesicles, with affinity for both K^+ and Na^+, then it would be expected that supply of 50 mM Na^+ together with 50 mM K^+ would produce a rate of antiport intermediate between that observed after supply of 100 mM Na^+ or 100 mM K^+. The data in Figure 5, however, show that in the presence of the 50 mM K^+ + 50 mM Na^+ mixture, antiport activity was higher than in that of either cation supplied alone at 100 mM. This result was obtained both when ΔpH was formed by supplying ATP to the membrane H^+-ATPase (Fig. 5A) and when it was imposed by the pH-jump technique (Fig. 5B). It suggests that separate antiporters for Na^+ and K^+ may be present.

The same conclusion is suggested by the fact that additive effects are obtained when saturating concentrations of Na^+ and of K^+ are supplied together. Figure 6A shows the dependence of Na^+ antiport and of K^+ antiport on the external concentration of the respective cation. A saturation curve relates ΔpH dissipation to cation concentration in both cases, but the plateau level for Na^+ antiport activity is 50% higher than that for K^+ antiport. The cation concentration for half saturation was also somewhat lower in the case of K^+ (32 mM as compared with 75 mM for Na^+) suggesting that both V_{max} and K_m may be lower, but this apparent higher affinity for K^+ was not always reproducible.

Figure 6B shows that even when the K^+ concentration was high enough to saturate K^+/H^+ antiport completely, addition of Na^+ produced further dissipation of the ΔpH. Conversely, Figure 6B also suggests that addition of K^+ to 200 mM Na^+, a Na^+ concentration high enough to saturate the Na^+/H^+ antiport (Fig. 6A), also enhanced ΔpH dissipation.

Attempts to measure the kinetics of the antiport in the absence of concomitant H^+-ATPase activity by applying the pH-jump technique gave somewhat variable results, possibly because the ΔpH is imposed on the whole population of

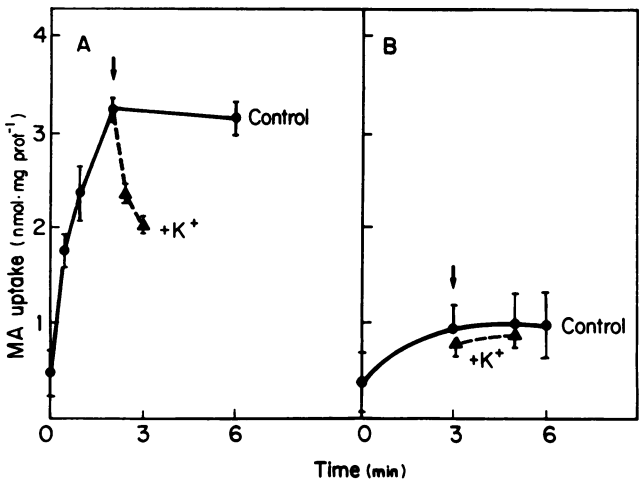

Figure 3. H^+-gradient-dependent methylamine (MA) uptake and release in membrane vesicles (fractions 6 and 7) isolated from cotton roots. (A) Vesicles prepared to contain BTP-MES buffer at pH 5.5, and diluted at time 0 into buffer at pH 7.0. (B) Vesicles prepared to contain BTP-MES buffer at pH 7.0, and diluted at time 0 into buffer at pH 7.0. Arrow indicates addition of KMes to final concentration of 20 mM. Medium as given in "Materials and Methods," BTP-Cl concentration 20 mM. Points give means of quadruplicates ± SE.

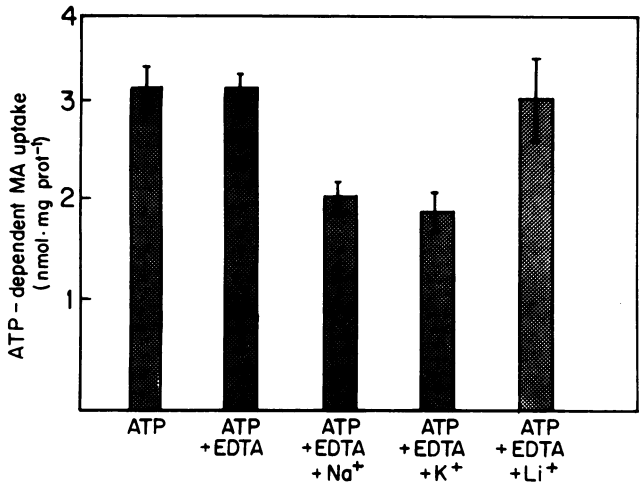

Figure 4. Effect of adding K^+, Na^+, or Li^+ together with EDTA, on the level of ATP-dependent methylamine (MA) accumulation in cotton root vesicles (fractions 6 and 7). ATP (5 mM) was added at time 0; the cations 10 min later; antiport was assessed after a further 30 s. Medium as given in "Materials and Methods," EDTA concentration 2 mM, BTP-Cl concentration 20 mM, cation concentration 20 mM. The points give the means of quadruplicates ± SE.

vesicles whether inside out, right-side out, possessed of antiport activity or not. In experiments where ATP was supplied to elicit ΔpH formation and the H^+-ATPase was subsequently inactivated by adding EDTA the treatment tended to cause the vesicles to become leaky to H^+, complicating interpretation of the results; when apyrase was used to stop the H^+-ATPase activity considerably slower H^+ leakage was observed.

Comparison of Salt-Grown and Non-Salt-Grown Atriplex Plants

Both Na^+/H^+ and K^+/H^+ antiport activity were detected even when the plants had been grown in the absence of added NaCl. Figure 7 gives Na^+/H^+ activity as a function of external Na^+ concentration. The pH-jump technique was used for this experiment to generate ΔpH, since the lower level of H^+-ATPase activity in non-salt-grown plants (6) complicates interpretation of experiments where ΔpH is ATPase-generated. Figure 7 shows that the level of antiport activity per unit membrane protein was lower for non-salt-grown than for salt-grown plants. Note, however, that the ΔpH created by the pH jump was about 40% lower in the case of the non-salt-grown plants (see Fig. 7 legend). When methylamine displacement is calculated as percentage of the methylamine accumulated in response to the pH jump (Fig. 7, values in parentheses) it is seen that this difference between non-salt-grown and salt-grown antiport activity manifests itself only at the lower NaCl concentrations; antiport activity from salt-grown plants seem to show positive cooperativity.

Location of the Antiporter

Most of the experiments reported above were, in the case of both plant species, carried out on a membrane fraction where ATP-dependent SCN^- accumulation is vanadate-sensitive, and stimulated by K^+ (6, 10). Methylamine accumulation in this fraction is NO_3^- stable, as illustrated in Figure 8 for cotton formation of ΔpH, and its subsequent dissipation by K^+, were scarcely affected by the presence of 50 mM NO_3^-. It is therefore likely that this fraction is plasmalemma-enriched. The H^+-ATPase in the lighter fraction (3, 4, and 5) is NO_3^- sensitive. For instance, in an experiment with cotton, 20 mM $BTPNO_3^-$ produced 60% inhibition of H^+-ATPase activity in this fraction, but no detectable inhibition in the plasmalemma-enriched fraction. It is likely that this lighter fraction is tonoplast-enriched, and we have obtained evidence in the case of both plants that cation/H^+ antiport is detectable here too. Figure 9 illustrates this point for *Atriplex*. H^+-Translocating activity was sensitive to NO_3^-; moreover, antiport activity was not observed in the presence of NO_3^- though it was manifested in its absence. The ΔpH dissipated by the cation had thus been built up by the NO_3^- sensitive ATPase, presumably the tonoplast ATPase.

DISCUSSION

Evidence has been obtained for Na^+/H^+ and K^+/H^+ exchange in the membrane vesicles isolated from both plants investigated here. Addition of the alkali cation, after a ΔpH had been established across the vesicle membrane, brought about dissipation of the ΔpH, but not of ΔΨ (7, 10). We have concluded that coupling of the opposed ion fluxes by a membrane component is the most likely explanation for the

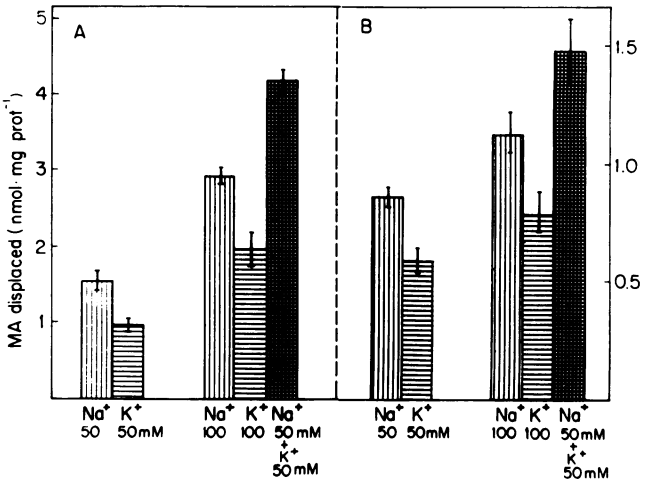

Figure 5. Antiporter activity in root membrane vesicles (fractions 6 and 7) isolated from salt-grown *Atriplex* plants, observed after supply of Na^+ and K^+ separately or together. (A) NaCl or KCl was added 10 min after 5 mM ATP was supplied to generate ΔpH, when intravesicular methylamine (MA) level was 9.5 ± 0.6 nmol/mg protein; antiport assessed after 1 additional min. Medium as given in "Materials and Methods," BTP-Cl concentration 100 mM. The points give the means of quadruplicates ± SE. (B) NaMes or KMes was added 3 min after vesicles, prepared so as to contain buffer pH 5.5 were transferred to pH 7.0 to impose a transmembrane ΔpH. Intravesicular methylamine level at time of cation addition 2.8 ± 0.2 nmol/mg protein; antiport assessed after 1 further min. Medium as given in "Materials and Methods," BTP-Cl concentration 40 mM. The points give the means of triplicates ± SE.

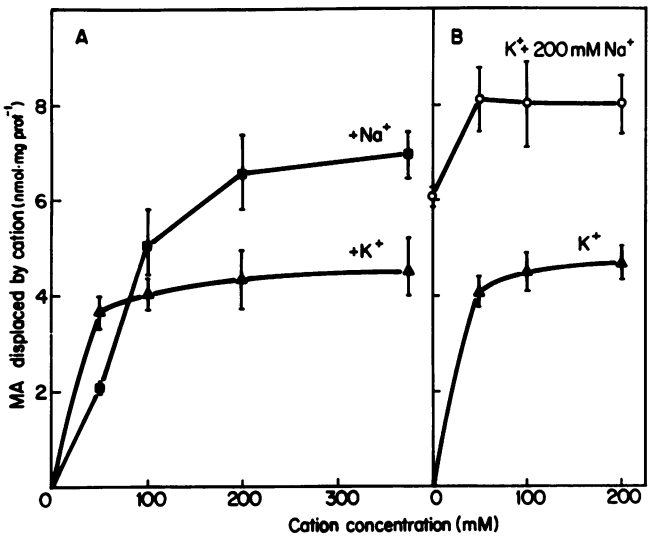

Figure 6. Dependence of antiporter activity in root membrane vesicles (fractions 6 and 7) on Na^+ or K^+ concentration supplied. The vesicles were isolated from salt-grown *Atriplex* plants. (A) NaCl or KCl was added 10 min after 5 mM ATP was supplied to generate ΔpH, when the intravesicular methylamine (MA) level was 7.4 ± 0.1 nmol MA/mg protein; antiport assessed after 1 further min. Medium as given in "Materials and Methods"; BTP-Cl concentration 100 mM. Points give means of quadruplicates ± SE. (B) Antiporter activity in the *Atriplex* vesicles in response to K^+ supplied alone, or supplied together with 200 mM Na^+. Intravesicular methylamine level at time of cation supply was 11.3 ± 0.8 nmol MA/mg protein. Other experimental details as for Figure 6A.

observed exchange; the alternative explanations which suggest themselves are inadequate for the following reasons: Mere electric, nonspecific exchange is very improbable since chloride was present at concentrations which completely depolarize the membrane potential. In several of the experiments, moreover, (see *e.g.* Fig. 8) 40 mM choline chloride was not only added to the external medium but was preloaded into the vesicle as well. The observed discrimination between cations, and the saturatable kinetics, also militate against electric coupling.

The fact that the antiport effect was observed when 40 mM choline (Fig. 8) was present inside and outside the vesicles also makes it very unlikely that the cation was merely displacing the positively charged pH probe from anionic membrane sites such as anionic lipids—the choline would be expected to compete very effectively with other cations, including the protonated form of methylamine and the alkali cations, for such sites, and thus to minimize adsorption of the probe to the membranes. Moreover, displacement of probe was considerably less in the absence of ΔpH, though the number of anionic sites would presumably not be affected. Displacement was also very slight in the absence of a ΔpH in pH jump experiments, *i.e.* when vesicles were transferred from pH 7.0 to pH 7.0 (as compared with pH 5.5–7.0).

The H^+ efflux was not the result of inhibition of the H^+-ATPase by the cations, since no inhibition could be detected at the cation concentrations used. On the contrary, stimulation was observed (7, 10). Moreóver, the H^+/cation exchange was also obtained when ΔpH is generated artificially by the pH jump technique and not by the H^+-ATPase (Fig. 3 for cotton; Fig. 5B and Fig. 7 for *Atriplex*).

The presence of antiporters is thus indicated in the membranes of the glycophyte cotton as well as those of the halophyte *Atriplex*. Reports of antiporter activity in glycophytes have so far been sparse (16, 17) and this investigation has provided the most detailed evidence so far obtained in favor of their presence. In the case of the halophyte, the level of Na^+/H^+ antiport was markedly higher than that of K^+/H^+ antiport over most of the concentration range examined. In cotton on the other hand, discrimination between Na^+ and K^+ was less clear (Li^+ was very poorly antiported, Fig. 4) but, in fact, K^+/H^+ antiporter activity was the highest in the concentration range examined (higher concentrations could not be used owing to their inhibiting effect on H^+-ATPase

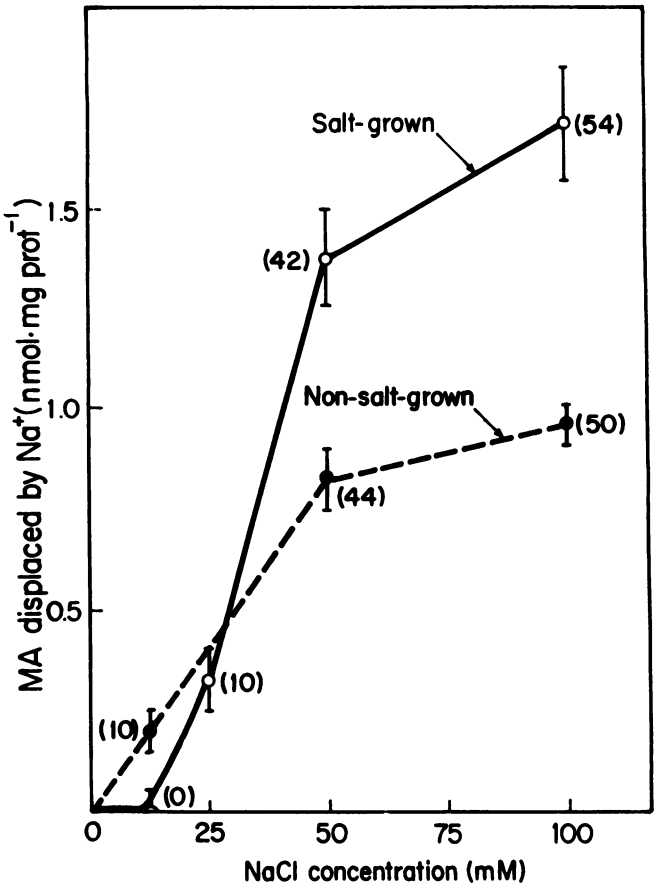

Figure 7. Na^+/H^+ antiporter activity as a function of Na^+ concentration in membrane vesicles isolated from the roots of salt grown or non-salt-grown *Atriplex* plants (fractions 6 and 7). NaCl was added to the medium 90 s after vesicles, prepared so as to contain buffer at pH 5.5, were diluted into buffer at pH 7.0 to impose a transmembrane ΔpH; antiport was assessed after 1 additional min. Intravesicular methylamine (MA) level at time of NaCl addition was 3.2 ± 0.2 or 1.9 ± 0.2 nmol/mg protein for salt-grown and non-salt-grown plants, respectively. Medium as given in "Materials and Methods," BTP-Cl concentration 40 mM. The points give the means of triplicates ± SE. Values in parentheses show methylamine displacement as a percent of methylamine accumulated in response to the pH jump.

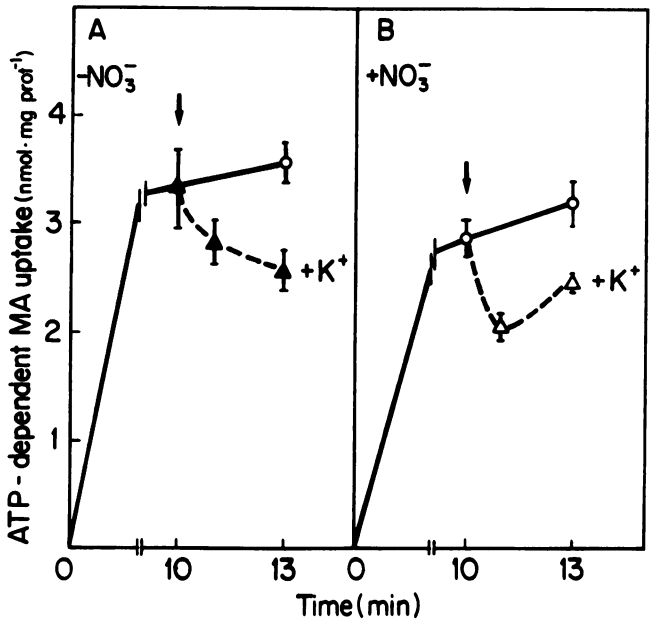

Figure 8. Effect of adding KMes on the level of ATP-dependent methylamine (MA) accumulation in cotton root membrane vesicles (fractions 6 and 7) in the absence (A) or presence (B) of 50 mM BTP-NO_3. The arrow indicates supply of K^+ to a final concentration of 20 mM. The vesicles were prepared to contain 40 mM choline Cl, which was also present at this concentration in the medium. The points give means of quadruplicates ± SE.

activity). Highly specific K^+/H^+ antiport has recently been observed in another glycophyte, *Brassica napus* (S Cooper, HR Lerner, L Reinhold, unpublished data).

The important question arises, are there in fact specific antiporters transporting each cation, or is only one antiporter involved, of rather low specificity? The evidence presented here for *Atriplex* (Figs. 5 and 6) indicating additive effects of Na^+ and K^+ even when both ions are presented at saturating concentrations, suggests that there are two antiporters. The situation thus recalls that in bacteria, where good evidence has accumulated for two antiporters (15). However, an alternative possibility is that in *Atriplex* there is one common antiporter, but that its performance is strongly modified when both Na^+ and K^+ are bound.

Na^+/H^+ antiport activity in *Atriplex* appeared to be modified by growing the plants in the presence of 400 mM NaCl. It was present, however, even in the nonsalt-grown plants, indicating that the system is constitutive in *Atriplex*. Saline growth conditions perhaps mainly modify transport kinetics rather than induce additional synthesis of the antiporter (as suggested for beet cells in suspension culture [2]) or induce its activation (as seems to be the case for a salt-tolerant barley variety [9]).

This investigation has produced evidence for the presence of antiporters both in the plasmalemma and in the tonoplast. The previous reports of antiporter activity in higher plant cells have been largely confined to tonoplast vesicles (1, 2, 9, 13). Evidence that antiporters are also located in the plasmalemma in the two plants investigated here may be seen in the fact that a ΔpH was built up in the plasmalemma-enriched fraction in the presence of 50 to 100 mM NO_3^-, and that this NO_3^--stable ΔpH was dissipated on addition of K^+ or Na^+ (Fig. 8) (7). In the case of the tonoplast-enriched fraction establishment of a ΔpH was severely depressed by NO_3^- and no antiport was detectible in its presence (Fig. 9).

A role for a Na^+ antiporter in both tonoplast and plasmalemma is readily apparent. Transfer of Na^+ into the vacuole at the tonoplast in exchange for protons, and ejection of Na^+ from the cell at the plasmalemma, would assist in controlling Na^+ concentration in the cytoplasm, especially in the case of the halophyte exposed to high ambient Na^+ concentrations. Transfer of K^+ into the vacuole by a K^+/H^+ antiporter driven by the outwardly directed protonmotive force across the tonoplast would also serve an obviously useful purpose. At the plasmalemma the K^+/H^+ antiporter might be regarded as a device for controlling the reentry of extruded protons into the cell and thus contributing substantially to internal pH control (see literature for bacterial cells, *e.g.* 15). Booth (3) has suggested that in the whole cell the cation/H^+ antiporters involved in pH homeostasis are regulated via binding of low mol wt ligands, or alternatively, by covalent protein modification. Such control mechanisms probably do not function

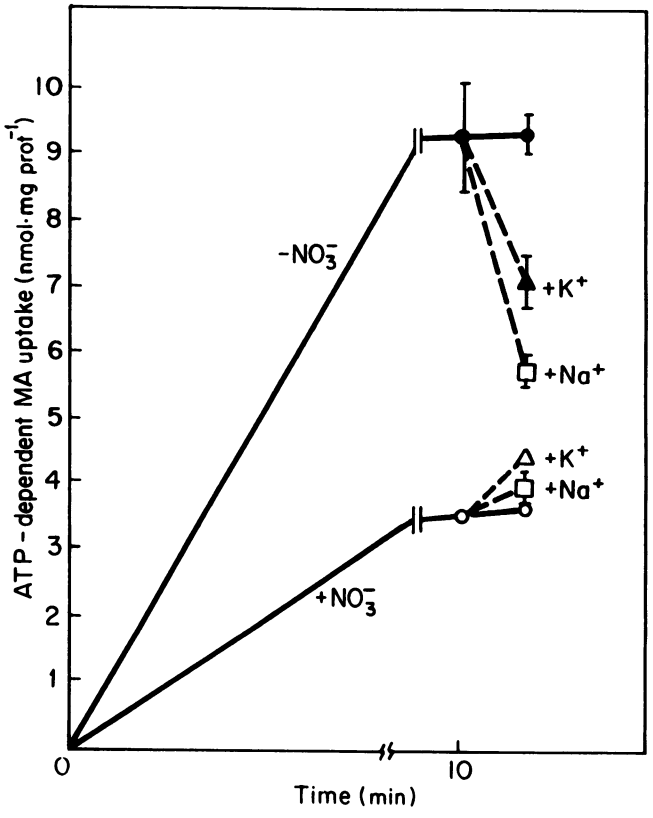

Figure 9. Effect of adding NaMes or KMes on the level of ATP-dependent methylamine (MA) accumulation in *Atriplex* root membrane vesicles (fractions 3, 4, and 5) in the presence or absence of 100 mM BTP-NO_3^-. The *Atriplex* plants were salt-grown. The arrow indicates supply of the cation to a final concentration of 100 mM. Medium as given in "Materials and Methods," BTP-Cl concentration 100 mM. The points give means of quadruplicates ± SE.

in isolated vesicles due to loss of components during their isolation.

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