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Sodium-Dependent Nitrate Transport at the Plasma Membrane of Leaf Cells of the Marine Higher Plant *Zostera marina* L.[1]

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NO_3^- is present at micromolar concentrations in seawater and must be absorbed by marine plants against a steep electrochemical potential difference across the plasma membrane. We studied NO_3^- transport in the marine angiosperm *Zostera marina* L. to address the question of how NO_3^- uptake is energized. Electrophysiological studies demonstrated that micromolar concentrations of NO_3^- induced depolarizations of the plasma membrane of leaf cells. Depolarizations showed saturation kinetics (K_m = 2.31 ± 0.78 μM NO_3^-) and were enhanced in alkaline conditions. The addition of NO_3^- did not affect the membrane potential in the absence of Na^+, but depolarizations were restored when Na^+ was resupplied. NO_3^--induced depolarizations at increasing Na^+ concentrations showed saturation kinetics (K_m = 0.72 ± 0.18 mM Na^+). Monensin, an ionophore that dissipates the Na^+ electrochemical potential, inhibited NO_3^--evoked depolarizations by 85%, and NO_3^- uptake (measured by depletion from the external medium) was stimulated by Na^+ ions and by light. Our results strongly suggest that NO_3^- uptake in *Z. marina* is mediated by a high-affinity Na^+-symport system, which is described here (for the first time to our knowledge) in an angiosperm. Coupling the uptake of NO_3^- to that of Na^+ enables the steep inwardly-directed electrochemical potential for Na^+ to drive net accumulation of NO_3^- within leaf cells.

Zostera marina L. is an aquatic angiosperm that grows in a medium with a high salinity—seawater itself—with NaCl concentrations in the region of 0.5 M. Leaf cells of this plant exhibit a plasma membrane potential (E_m) of around −160 mV in natural seawater (Fernández et al., 1999). Molecular and physiological evidence indicates that the E_m in this halophyte is maintained by the activity of a H^+-pump (Fukuhara et al., 1996; Fernández et al., 1999). With this highly negative E_m, the uptake of essential anions such as NO_3^-, which usually occurs in seawater at concentrations of 1 to 500 μM (Riley and Chester, 1971) and is probably below 10 μM in the close environment of the plant (Hernández et al., 1993), must be energized.

Most studies on vascular plants and algae have reported that NO_3^- transport is powered by the electrochemical potential for protons present across the plasma membrane of plant cells (Ullrich, 1992). Evidence includes simultaneous measurements of NO_3^- and H^+ fluxes (Mistrik and Ullrich, 1996), NO_3^--evoked membrane depolarizations (McClure et al., 1990; Ullrich and Novacky, 1990; Glass et al., 1992), and pH dependence of NO_3^--elicited inward currents both in intact plants (Meharg and Blatt, 1995) and in oocytes expressing plant NO_3^- transporters (Tsay et al., 1993; Zhou et al., 1998; Liu et al., 1999). Four different NO_3^- uptake systems have been identified in higher plants: both low- and high-affinity systems are present and can be constitutive or inducible (Wang and Crawford, 1996; Liu et al., 1999). High-affinity systems show saturation kinetics and operate at concentrations lower than 0.5 mM with K_m values in the range of 7 to 100 μM; low-affinity systems show linear kinetics and function at concentrations above 0.5 mM (Wang and Crawford, 1996; Crawford and Glass, 1998).

Energization of solute transport by H^+ coupling is prevalent among bacteria, fungi, and plants. Nevertheless, for the plasma membrane of most cells the presence of an inwardly directed electrochemical potential difference for Na^+ can potentially be exploited for energization of solute transport through Na^+ coupling. Accordingly, a number of Na^+-dependent transport systems have been identified. Na^+-dependent uptake of Glc and amino acids has been shown in the marine diatom *Cyclotella* (Hellebust, 1978), and phosphate transport has been reported to be stimulated by Na^+ in several green algae (Raven, 1984). In the case of NO_3^-, Na^+-dependent uptake has been described only in the marine diatom *Phaeodactylum tricornutum* (Rees et al., 1980) and in cyanobacteria (Lara et al., 1993).

During the past decade an emerging number of Na^+-coupled transport systems have also been discovered at the plasma membranes of multicellular plants. In freshwater charophyte algae, the influx of K^+, urea, and Lys has been shown to be Na^+ dependent and/or directly coupled to the transport of Na^+ (Smith and Walker, 1989; Walker and Sanders, 1991), and Na^+-dependent K^+ uptake has also been demonstrated in some aquatic angiosperms such as *Egeria*, *Elodea*, and *Vallisneria* (Walker, 1994; Maathuis et al., 1996). A Na^+,K^+ symporter, HKT1, has been cloned from wheat (Rubio et al., 1995), but it remains unclear whether

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Na^+ coupling comprises the dominant mode of energizing K^+ uptake in terrestrial angiosperms (Maathuis et al., 1996).

The elevated Na^+ concentration present in seawater and the highly negative E_m in *Z. marina* makes feasible the existence of this type of alternative transport system powered by the putative electrochemical potential for Na^+. To test this hypothesis, we have investigated NO_3^- transport at the plasma membrane of leaf cells of *Z. marina* to determine the possible interactions between Na^+ and NO_3^-. These interactions have been studied with respect to the electrophysiological properties of the plasma membrane and to NO_3^- depletion in media surrounding whole leaves.

MATERIALS AND METHODS

Plant Material

Zostera marina L. plants were collected off the coast of Málaga (Spain) at a 5-m depth. Plants were maintained in the laboratory in natural seawater at 15°C and a light intensity of 150 μmol m^{-2} s^{-1}, with a photoperiod of 16 h of light and 8 h of darkness.

Plants were N-starved prior to experiments for at least 3 d in N-free artificial seawater (ASW) adjusted to pH 8.0 with NaOH. The composition of ASW was 0.010 mM KH_2PO_4, 1.2 mM $NaHCO_3$, 5 mM K_2SO_4, 12 mM $CaCl_2$, 15 mM $MgSO_4$, 42.5 mM $MgCl_2$, and 500 mM NaCl.

Membrane Potential Measurements

Leaf pieces were excised to remove partially the epidermis, and were mounted in a plexiglass chamber (volume 1.1 mL). Continuous perfusion of the assay medium was maintained at a flux of approximately 10 mL/min. Both epidermal and mesophyll cells were impaled with single-barrel electrodes. Membrane potentials were measured using the standard glass microelectrode technique as described by Felle (1981). Micropipettes were backfilled with 0.5 M KCl. Microelectrodes were fixed to electrode holders containing an Ag/AgCl pellet, and connected to a high-impedance differential amplifier (FD-223, World Precision Instruments, Sarasota, FL). Tip potentials never exceeded −10 mV.

Experiments were carried out in N-free ASW (as above) buffered with 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES)-Tris(hydroxymethyl)-aminomethane (Tris) to pH 8.0. NO_3^- was added as KNO_3. To check the effect of pH, the same buffer was adjusted to pH 6.0 or 7.0. To test the effect of Na^+, N-free ASW with a slightly different composition was used (NaCl-ASW: 2.5 mM $KHCO_3$, 2.5 mM K_2SO_4, 5 mM $CaSO_4$, 5 mM $MgSO_4$, 500 mM NaCl, and 10 mM HEPES-Tris). In Na^+-free ASW, NaCl was substituted by 0.8 M sorbitol (sorbitol-ASW). Na^+ was added to the medium at increasing concentrations as Na_2SO_4 or NaCl.

NO_3^- Depletion Experiments

Whole plants were maintained for 3 d in N-free ASW and were preincubated in the assay medium for 1 h before starting the experiment. The composition of assay medium was the same as for impalements. Whole leaves (0.6–0.9 g fresh weight) were incubated in 100-mL flasks. Three replicates were assayed for each treatment (NaCl-ASW, sorbitol-ASW, sorbitol-ASW + 5 mM Na_2SO_4, and sorbitol-ASW + 10 mM NaCl). The assay was carried out with slow and constant agitation at 25°C and at a light intensity of 50 μmol m^{-2} s^{-1}. The first treatment (NaCl-ASW) was also assayed in darkness. KNO_3 was added at an initial concentration of 100 μM. Samples were taken at 0, 1, 2, 4, 8, 12, and 24 h. NO_3^- was analyzed colorimetrically as described in García-Sánchez et al. (1993).

Chemicals

pH buffers and sorbitol were from Sigma-Aldrich (St. Louis). Monensin (Sigma) was dissolved in ethanol at a concentration of 50 mM.

Data Analysis

Data are given as means ± SE. Membrane depolarization data were fitted with the Michaelis-Menten equation using a non-linear regression computer program (KaleidaGraph, Synergy Software, Reading, PA).

RESULTS

Effect of NO_3^- Additions on the Membrane Potential

Figure 1A demonstrates that NO_3^- evoked a rapid membrane depolarization in N-starved plants in the light. Measurable changes in E_m were evident even at 1 μM NO_3^- (not shown). When NO_3^- was washed from the medium, the membrane first hyperpolarized so that E_m was transiently more negative than the original resting potential, and then depolarized with another overshoot to a value

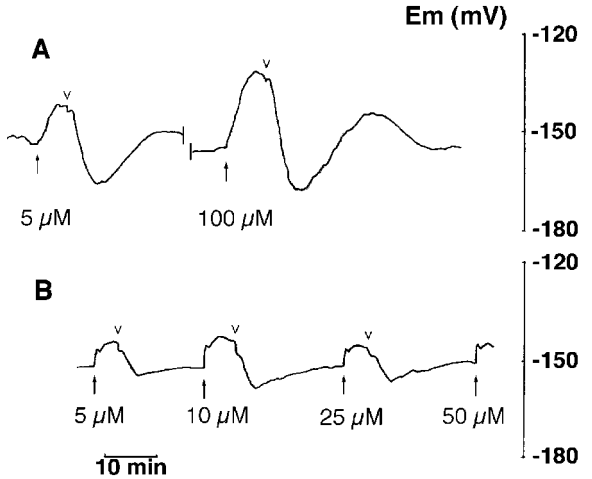

Figure 1. Effect of the addition of NO_3^- on E_m. A, Tissue in the light, incubated in ASW, showing the response of a single mesophyll leaf cell to the addition of 5 and 100 μM KNO_3. B, Tissue in the dark with KNO_3 added at the concentrations indicated. Small downward arrowheads indicate the onset of the NO_3^- wash.

close to that before NO_3^- addition (Fig. 1A). Depolarizations showed saturation at concentrations around 50 μM NO_3^- (Fig. 2). Because depolarization is an integral function of the net charge carried by the NO_3^- transport system, the concentration dependence of the depolarization can be taken as a rough guide to the affinity of this system for its substrate. Fitting the data with the Michaelis-Menten equation yielded a K_m value of 2.31 ± 0.78 μM NO_3^- and a maximum depolarization of 15.6 ± 0.9 mV (Fig. 2).

NO_3^--induced depolarizations were lower in the dark than in the light, with maximum values around 10 mV, and they did not exhibit saturation kinetics (Figs. 1B and 2). The response of E_m to NO_3^- additions was also different (Fig. 1B): the depolarization was very rapid and when NO_3^- was washed from the medium the E_m did not exhibit the overshoot in the depolarizing direction before returning to the resting value.

The depolarizations elicited by NO_3^- suggest that uptake of this anion involves flow of positive charge into the cell.

Effect of pH on NO_3^--Induced Depolarizations

Many H^+-coupled transport systems in plants are activated by a rise in external H^+ concentration (Bush, 1993), and this enhancement of electrophoretic transport can be reflected in a larger transport-related depolarization as the pH is lowered. We therefore examined the pH dependence of the NO_3^--induced depolarization. In contrast to many H^+-coupled transport systems, the depolarizations induced by saturating NO_3^- concentrations (100 μM) were lower at acidic than at alkaline pH (Fig. 3). A shift of external pH from 8.0 to 6.0 decreased the magnitude of the depolarization by a factor of about 3.

Effect of Na^+ on NO_3^--Evoked Depolarizations

To determine whether Na^+ was involved as the coupling ion, we examined the capacity of NO_3^- to depolarize the membrane in the absence of Na^+. Replacement of NaCl in the medium with isosmotic sorbitol produced a slight hyperpolarization of the membrane (data not shown). In Na^+-free conditions, NO_3^--induced membrane depolarizations were abolished (Fig. 4). The addition of Na^+ (as Na_2SO_4) at concentrations as low as 250 μM to NaCl-free ASW (sorbitol-ASW) restored the NO_3^--induced depolarizations observed in NaCl-ASW (Figs. 4 and 5). In contrast, NO_3^- additions in the presence of KCl did not produce any effect (Fig. 4) and depolarizations were of the same order when Na^+ was added as NaCl or Na_2SO_4 (data not shown).

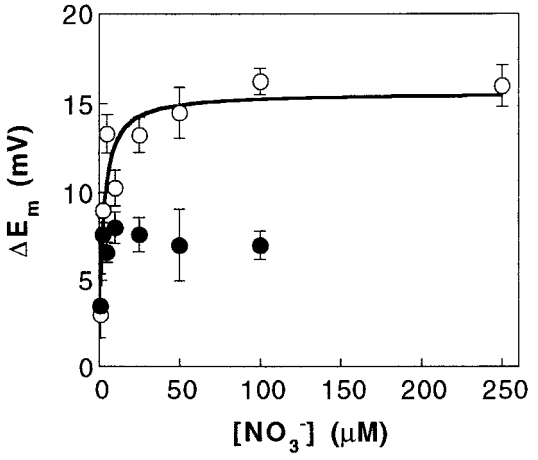

Figure 2. Membrane depolarizations induced by increasing the NO_3^- concentration. Mesophyll leaf cells were impaled in ASW in the light (○) or in the dark (●). Data are the means ± SE of three independent replicates. Values in the light were fitted with the Michaelis-Menten relationship, as shown by the curve.

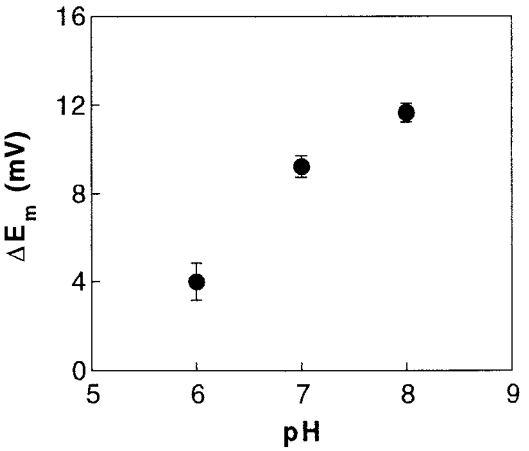

Figure 3. Effect of pH on NO_3^--induced depolarizations. Mesophyll leaf cells were impaled in ASW buffered at different pHs and challenged with saturating NO_3^- concentrations (100 μM KNO_3). Values are the means ± SE of five independent replicates.

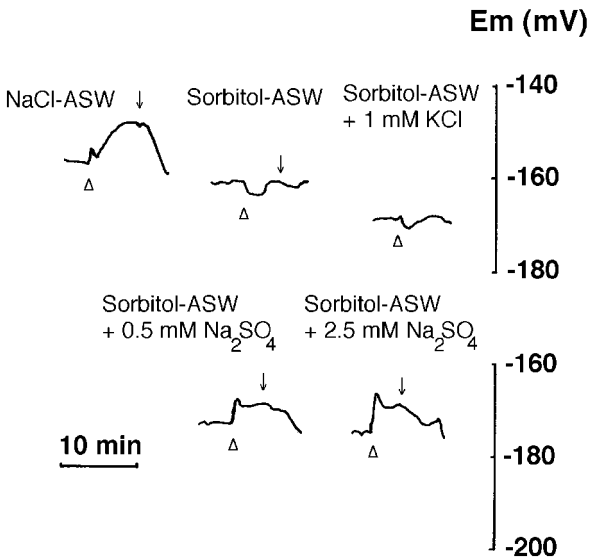

Figure 4. NO_3^--induced depolarizations in the presence and absence of Na^+. Lines show the response of E_m of a single mesophyll leaf cell to 100 μM KNO_3 addition (△). The tissue was incubated sequentially in different media: ASW containing NaCl (NaCl-ASW), NaCl-free ASW (sorbitol-ASW), and sorbitol-ASW to which KCl or Na_2SO_4 were added as indicated. Downward arrows indicate onset of NO_3^- wash.

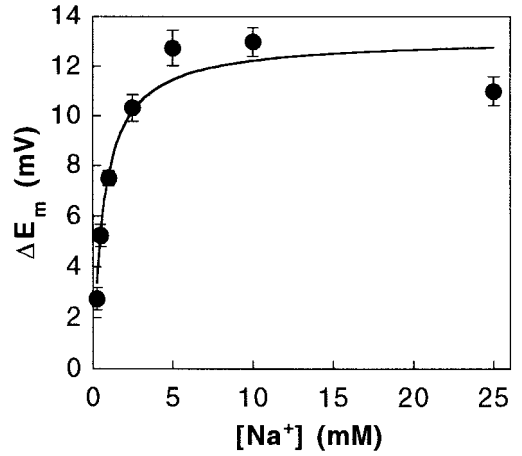

Figure 5. Effect of increasing Na^+ concentrations on NO_3^--induced depolarization. Leaf cells were incubated in NaCl-free ASW (sorbitol-ASW) and challenged with saturating NO_3^- concentrations (100 μM KNO_3). Na^+ was added as Na_2SO_4. The data are the means ± SE of three independent replicates. The solid line represents a non-linear least squares fit of the data to the Michaelis-Menten equation.

The kinetics with respect to Na^+ ions was further investigated, with depolarization in response to saturating NO_3^- concentrations (100 μM) monitored over a range of Na^+ concentrations (Fig. 5). The results demonstrate that the response is effectively saturated at around 5 mM Na^+. Fitting of the data with the Michaelis-Menten equation yielded a K_m of 0.72 ± 0.18 mM Na^+ and a maximum depolarization of 13.1 ± 0.7 mV.

Effect of Monensin on NO_3^--Induced Depolarizations

The ionophore monensin, which dissipates the electrochemical potential for Na^+ (Pressman, 1976), was added to the assay medium at a concentration of 50 μM to determine its effects on the NO_3^--induced depolarization. The presence of monensin in absence of NO_3^- failed to evoke significant changes in E_m (data not shown). However, monensin partially inhibited the depolarization induced by NO_3^- (Fig. 6), showing a mean inhibition value in tests with 100 μM NO_3^- of 85.0% ± 20.7% (n = 8).

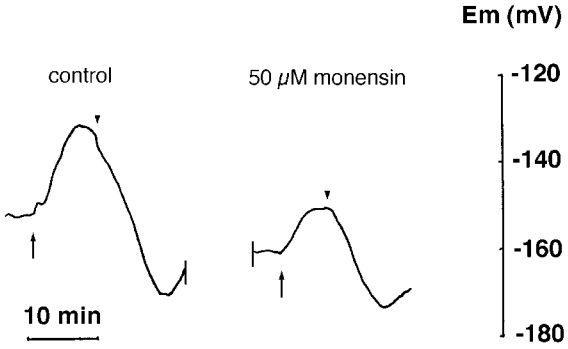

Figure 6. Response of E_m of a single mesophyll leaf cell to the addition of 100 μM KNO_3 (indicated by the long upward arrows) incubated in ASW before (control) and after the addition of 50 μM monensin. ▼, Onset of NO_3^- wash.

NO_3^- Uptake Rates in the Absence and Presence of Na^+

If NO_3^- transport in *Z. marina* is Na^+ coupled, then net uptake from the medium should be enhanced in the presence of Na^+. Table I shows that NO_3^- was depleted from the medium by leaves at higher rates when Na^+ was present. NO_3^- uptake rates were measured in NaCl-free ASW (sorbitol-ASW) and in the same medium but with the addition of 10 mM Na^+ as Na_2SO_4 or NaCl. The differences between the mean NO_3^- uptake rates in the presence and absence of Na^+ were significant (t test, $P < 0.05$), and the addition of Na^+ more than doubled the net NO_3^- uptake rate. Furthermore, there were no significant differences between the means obtained in the presence of Na_2SO_4 or NaCl.

The uptake of NO_3^- was also measured in ASW containing 0.5 M NaCl in the light and in the dark. The difference between the mean values was significant (t test, $P < 0.1$), being 2-fold higher in the light than in the dark (Table I).

DISCUSSION

The depolarizations induced by NO_3^- in *Z. marina* indicate that the uptake of this anion is coupled with the inward movement of positive charge. However, since the magnitude of the depolarization is decreased at acid pH, it appeared possible that in this plant NO_3^- uptake is not coupled with the entrance of H^+ as is the rule in other angiosperms (Ullrich, 1992). Nevertheless, the decrease in depolarization observed at low pH might be explained by a direct pH effect on transporter kinetics or by an increase in membrane conductance.

In contrast, the lack of any NO_3^--induced depolarization in Na^+-free ASW and the restoring of the depolarization when Na^+ is added to the medium suggest that NO_3^- uptake into leaf cells is coupled with the entrance of Na^+. The stimulation of NO_3^- uptake rates by the leaves in the presence of Na^+ points to the same conclusion, as does the inhibition of NO_3^--evoked depolarizations in the presence of monensin, an ionophore that dissipates the Na^+ electrochemical gradient. However, because monensin, like other cation-H^+ exchangers, acts as an uncoupler of photosyn-

Table I. *Effect of Na^+ on NO_3^- uptake rates*

Whole leaves were incubated in different assay media: ASW containing 0.5 M NaCl (NaCl-ASW) or ASW in which NaCl was substituted with 0.8 M sorbitol (sorbitol-ASW). Additions of Na^+ were made to sorbitol-ASW as shown. NO_3^- uptake rates were measured in the light except where indicated. The values shown are the means ± SE of three replicates of a representative experiment.

Treatment	NO_3^- Uptake Rate
	$\mu mol\ NO_3^-\ g^{-1}\ fresh\ wt\ h^{-1}$
Sorbitol-ASW	0.12 ± 0.00
Sorbitol-ASW + 5 mM Na_2SO_4	0.35 ± 0.06
Sorbitol-ASW + 10 mM NaCl	0.28 ± 0.05
NaCl-ASW, light	0.62 ± 0.09
NaCl-ASW, dark	0.34 ± 0.05

thesis and respiration (Rottenberg, 1977), the ionophore might also affect NO_3^- transport indirectly via an effect on nitrogen metabolism.

Na^+-coupled NO_3^- transport has been demonstrated previously only in the marine diatom *Phaeodactylum tricornutum* (Rees et al., 1980) and in cyanobacteria, where it has been well defined (Lara et al., 1993). Calculated K_m values for NO_3^- (2.31 ± 0.78 μM) and Na^+ (0.72 ± 0.18 mM) in *Z. marina* are quite similar to the values reported in the cyanobacterium *Anacystis nidulans*, which exhibits a K_m for NO_3^- of 1.6 ± 0.2 μM and for Na^+ of 0.36 ± 0.04 mM (Rodríguez et al., 1994). The K_m for Na^+ in *Z. marina* is also close to the value reported for the marine diatom (2.58 ± 0.56 mM, Rees et al., 1980). These low-millimolar K_m values for Na^+ imply that the NO_3^- transporter of *Z. marina*, as well as those of the other organisms, will be functioning at saturating Na^+ concentrations in a marine environment. It should be noted, though, that the K_m for Na^+ reported in the present study was derived at a saturating concentration of NO_3^-, and that a rise in K_m as the concentration of the substrate ion is lowered cannot be precluded (Sanders et al., 1984).

Other features of NO_3^- transport are similar in *Z. marina* and cyanobacteria. NO_3^- uptake in cyanobacteria is inhibited by 60% in the presence of 25 μM monensin (Rodríguez et al., 1992); in *Z. marina*, 50 μM monensin produces an inhibition of the NO_3^--induced depolarizations of about 85%. Moreover, a cyanobacterium mutant lacking NO_3^- reductase activity shows an inhibition of NO_3^- uptake at concentrations around 25 μM (Rodríguez et al., 1994). In *Z. marina* NO_3^--evoked depolarizations, as well as NO_3^- uptake, are smaller in the dark and depolarizations reach saturation at lower concentration values than in the light. The lower NO_3^- uptake rate in the cyanobacterium mutant is explained by apparent substrate inhibition of the transporter (Rodríguez et al., 1994), as NO_3^- cannot be reduced. This effect might also be invoked here to explain the decrease in NO_3^- transport in the dark observed in *Z. marina*, as it is known that NO_3^- reductase activity declines in the absence of light mainly due to a lack of reducing power (Beevers and Hageman, 1980).

Maximum NO_3^--induced depolarizations measured in *Z. marina* are of the same order as the values reported in Arabidopsis root hairs (Meharg and Blatt, 1995; Wang and Crawford, 1996). The response of E_m when NO_3^- is washed is more complex in *Z. marina* than in Arabidopsis root hairs, and it is different in the light and in the dark. This suggests a role of N metabolism in determining the effect of NO_3^- additions on E_m (Mistrik and Ullrich, 1996) that could be more important in the light and in photosynthetically active tissue.

A kinetic study of Na^+-dependent NO_3^- transport in *A. nidulans* indicates that Na^+ and NO_3^- ions are the real substrates of the transporter and a minimum stoichiometry of one Na^+ per NO_3^- and a maximum of two has been proposed (Rodríguez et al., 1994). In *Z. marina*, the stoichiometry must be >1, since we have recorded membrane depolarization in response to NO_3^-.

Z. marina, as an angiosperm, evolved from terrestrial species that presumably, like extant species, transported NO_3^- from the soil using H^+-coupled transport systems. Therefore the question arises as to why Na^+-coupled NO_3^- transport evolved in *Z. marina*. This question might best be addressed by considering the thermodynamic aspects of cation-coupled NO_3^- transport in a marine environment, and that requires knowledge of the respective NO_3^-, Na^+, and H^+ electrochemical potentials across the plasma membrane. Cytosolic concentrations of NO_3^- ($[NO_3^-]_c$) and Na^+ ($[Na^+]_c$) have been estimated in some marine plants, showing a range for Na^+ of 1 to 50 mM and for NO_3^- of 0 to 1 mM (Raven, 1984). More accurate cytosolic measurements in non-marine plants give values of 3 to 5 mM for NO_3^- in barley root epidermal cells (Walker et al., 1995; van der Leij et al., 1998) and 50 mM for Na^+ in a salt-tolerant *Chara* species (Kiegle and Bisson, 1996). (Although total internal Na^+ concentrations have been estimated in two species of seagrass, being around 100 mM in epidermal leaf cells and showing higher values in other tissues [Beer et al., 1980], such estimates will reflect primarily the composition of the large central vacuole rather than that of the cytosol.) We might therefore take as reasonable estimates of $[NO_3^-]_c$ and $[Na^+]_c$ in *Z. marina* respective values of 3 and 50 mM. The cytosolic pH has been measured as 7.3 in leaf cells of this species (Fernández et al., 1999) and the membrane potential is typically −160 mV. Normal seawater concentrations of NO_3^- and Na^+ can be taken as 10 μM and 550 mM, respectively, and the pH is typically 8.0. The generalized free energy relationship for a plasma membrane cation-NO_3^- symporter operating with a stoichiometry of n cations per NO_3^- is given (in millivolts) as

$$\Delta G'/F = (n - 1)E_m + 59 \cdot \log[([C^+]_c^n \cdot [NO_3^-]_c)/([C^+]_o^n \cdot [NO_3^-]_o)] \quad (1)$$

where C^+ is the coupling cation (either Na^+ or H^+) and subscripts o and c denote the external solution and cytoplasm, respectively. Figure 7 shows the resulting free energy relationships for hypothetical cation-NO_3^- symporters operating in conditions appropriate to *Z. marina*.

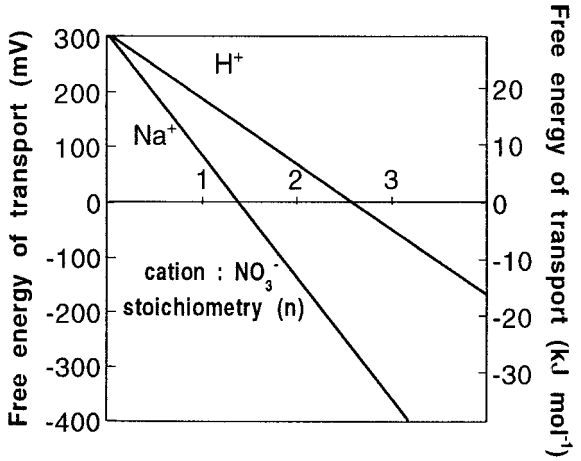

Figure 7. Thermodynamic relationship of hypothetical NO_3^- transporters employing either H^+ or Na^+ as the coupling ion. Calculations were performed with Equation 1, with parameter values assigned as discussed in the text.

Clearly, in the case of H^+ coupling, a stoichiometry of two is not sufficient to drive net uptake (free energy > 0), and even a stoichiometry of three yields only a modest inward driving force of -5 kJ mol^{-1}. It is likely that a still higher value of *n* would be required to guarantee NO_3^- influx in varying external conditions. By contrast, Na^+-coupled transport would be strongly inwardly directed (-13 kJ mol^{-1}), with a stoichiometry of just $2Na^+:NO_3^-$ (Fig. 7).

NO_3^- transport kinetics in *Z. marina* indicates the existence of a high-affinity transport system, with a much lower K_m (2.31 ± 0.78 μM) than has to date been reported for other angiosperms (where values reside in the range 6–20 μM, Crawford and Glass, 1998), although the value in *Z. marina* is in the same range (2–13 μM) as that described for marine algae (DeBoer, 1985), the fungus *Aspergillus nidulans* (1 μM, A.J. Miller, personal communication), and cyanobacteria (Rodríguez et al., 1994). The low K_m observed in *Z. marina* is very likely to be an adaptation to an environment in which NO_3^- concentrations are typically <10 μM.

In higher plants, two high-affinity systems for NO_3^- have been defined: one inducible and another one constitutive (Wang and Crawford, 1996; Crawford and Glass, 1998). We have found that the E_m response to NO_3^- addition in N-starved plants does not show any lag, which might indicate the existence of a constitutive high-affinity system. However, we have also detected an increase in the magnitude of depolarizations after repeated additions of NO_3^- (data not shown), which could be the manifestation of an inducible transport system.

In conclusion, several lines of evidence indicate that a Na^+-dependent high-affinity NO_3^- transport system operates in *Z. marina*. This is the first report of Na^+-dependent NO_3^- transport in an angiosperm and points to the potential relevance of Na^+-coupled transport in halophytic species. The Na^+ dependence of NO_3^- transport in *Z. marina* also suggests that other anions could enter the cell using a similar mechanism, and studies of other anion transporters are now in progress.

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