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Studies of the Uptake of Nitrate in Barley

I. Kinetics of $^{13}NO_3^-$ Influx

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ABSTRACT

$^{13}NO_3^-$ was used to investigate patterns of NO_3^- influx into roots of barley plants (*Hordeum vulgare* L. cv Klondike) previously grown with ('induced') or without ('uninduced') a source of external NO_3^- ($[NO_3^-]_0$). In both induced and uninduced plants, $^{13}NO_3^-$ influx was biphasic in the range from 0.005 to 50 moles per cubic meter $[NO_3^-]_0$. In the low concentration range (<1 mole per cubic meter for induced plants and <0.3 mole per cubic meter for uninduced plants), influx was saturable and V_{max} and K_m values for influx either increased or decreased according to NO_3^- pretreatment. By contrast, $^{13}NO_3^-$ influx in the high concentration range revealed a strictly linear concentration dependence. These fluxes appeared to be mediated by a constitutive, rather than an inducible, transport system.

Studies of *net* NO_3^- uptake over a wide range of external NO_3^- concentrations ($[NO_3^-]_0$) have revealed that net NO_3^- uptake by plants previously grown with a source of NO_3^-, (and hence 'induced' for NO_3^- uptake) is biphasic (4, 24). At low $[NO_3^-]_0$, uptake occurs by a high affinity, saturable system, while at high concentrations (≥1 mol m^{-3} $[NO_3^-]_0$), uptake may be either saturable (4) or nonsaturable (4, 24) according to species or genotype. However, most studies on the kinetic and energetic aspects of NO_3^- uptake have been confined to the low-concentration system (2, 6 for review) and largely based upon net uptake studies. Net uptake studies should be interpreted with caution because they are the resultant of two separate fluxes, namely influx and efflux, and these processes may be mediated by two distinct transport systems. It is only recently that radioactive tracers ($^{13}NO_3^-$ and $^{36}ClO_3^-$) have been used to measure unidirectional NO_3^- fluxes with a view to probing the phenomena of induction and regulation of NO_3^- uptake (3, 8, 11, 18, 20, 23, 29, and references therein).

There is virtually no information available regarding the high concentration system nor are the characteristics of the uninduced systems clearly established. The present communication details the kinetic properties of the low- and high-concentration systems for NO_3^- influx, and their capacity for induction and negative feedback control by use of $^{13}NO_3^-$.

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MATERIALS AND METHODS

Seed Germination and Plant Growth

Seeds of barley (*Hordeum vulgare* L. cv Klondike) were germinated in sand moistened with distilled water for 3 d, and then transferred to hydroponic tanks containing modified 1/80th strength Johnson's nutrient solution with or without NO_3^-, as described previously (29).

NO_3^- Pretreatments

Effects of four different NO_3^- pretreatments on the kinetics of NO_3^- influx were investigated in 7 d old plants. The treatments were referred to as (a) uninduced plants: after 3 d of germination in sand, the seedlings were maintained in modified 1/80th strength Johnson's solution without nitrogen for 4 d, (b) 1-d-induced plants: after 3 d of germination in sand, seedlings were grown in modified 1/80th strength Johnson's solution without nitrogen for 3 d and with 0.1 mol m^{-3} NO_3^- (as $Ca[NO_3]_2$) for 24 h prior to $^{13}NO_3^-$ influx determinations, (c) and (d) 4-d-induced plants: After 3 d of germination in sand, the seedlings were maintained in modified 1/80th strength Johnson's solution containing either 0.1 or 10 mol m^{-3} NO_3^-, respectively, (as $Ca[NO_3]_2$) for 4 d.

Measurement of Influx

$^{13}NO_3^-$ influxes into intact roots were measured from modified 1/80th strength Johnson's solutions containing NO_3^- (as $Ca[NO_3]_2$) in the concentration range from 0.005 to 50 mol m^{-3}, with pH adjusted to 6. Prior to 10 min influx periods, roots were prewashed for 5 min in an identical but nonradioactive solution. Influx was followed by a 2 min wash of intact roots in an identical but nonradioactive solution to remove $^{13}NO_3^-$ from the free space (see Siddiqi *et al.* [29] for details). Roots were then excised and counted immediately in a Packard γ-counter (Minaxi ∂, Auto-γ 5000 series). The roots were then weighed and their NO_3^- content determined as described by Siddiqi *et al.* (29).

All experiments were repeated either three or four times. Each treatment in each experiment was replicated four times,

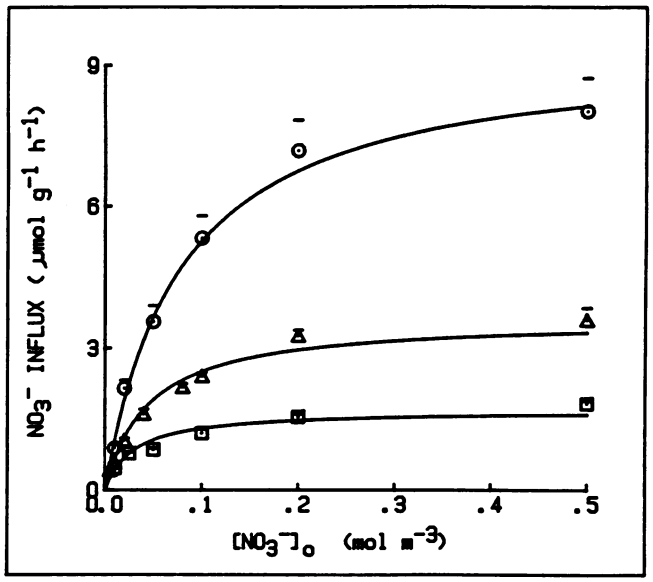

Figure 1. $^{13}NO_3^-$ influx from 0.005 to 0.5 mol m^{-3} $[NO_3^-]_o$ into roots of induced plants: plants pretreated with 0.1 mol m^{-3} NO_3^- for 1 d (○) or 4 d (△), or with 10 mol m^{-3} NO_3^- for 4 d (□) prior to influx determinations. See text for details. Horizontal bars represent standard errors of the means.

each replicate consisting of about 10 seedlings. The means and standard errors were calculated from values obtained from all the experiments (n = 12–16); thus each value represents a mean of 120 to 160 seedlings.

Production and Purification of $^{13}NO_3^-$

$^{13}NO_3^-$ was produced by the proton irradiation of H_2O on the TRIUMF-ACEL CP42 cyclotron using 20 MeV protons (29). ^{18}F, $^{13}NO_2^-$, and $^{13}NH_4^+$, present in the sample as contaminants, were removed as described by Siddiqi *et al.* (29).

RESULTS

The kinetic analyses of $^{13}NO_3^-$ influx into intact roots of 'induced' and 'uninduced' barley plants are shown in Figures 1 to 4 and discussed in detail below. These data demonstrate that $^{13}NO_3^-$ influx, in the concentration range from 0.005 to 50 mol m^{-3}, is mediated by two distinct transport systems in both induced and uninduced plants. At low external $[NO_3^-]$ ($[NO_3^-]_o$), $^{13}NO_3^-$ influx occurs by means of a high affinity, saturable system in both induced (Fig. 1) and uninduced (Fig. 2) plants. At high $[NO_3^-]_o$, the $^{13}NO_3^-$ influx system was characterized by low affinity for NO_3^- and a linear (nonsaturable) dependence on $[NO_3^-]_o$ in induced (Fig. 3) and uninduced plants (Fig. 4).

Low-Concentration System

In plants induced for nitrate uptake by pretreatment in 0.1 or 10 mol m^{-3} NO_3^- solutions, $^{13}NO_3^-$ influx displayed typical Michaelis-Menten kinetics (Fig. 1) in the concentration range from 0.005 to 0.5 mol m^{-3} $[NO_3^-]_o$. Influx saturated at about 0.2 to 0.5 mol m^{-3} $[NO_3^-]_o$ and the V_{max} for influx was negatively correlated with the duration of NO_3^- pretreatment and the NO_3^- concentration of the pretreatment solution (Fig. 1). Measurements of tissue $[NO_3^-]$ revealed that increasing the duration of exposure to 0.1 mol m^{-3} $[NO_3^-]$ (from 1 to 4 d) increased root $[NO_3^-]$ from about 40 to about 76 μmol g^{-1}. Plants exposed to 10 mol m^{-3} $[NO_3^-]$ for 4 d had a root $[NO_3^-]$ of 97 μmol g^{-1}. As root $[NO_3^-]$ increased V_{max} values for $^{13}NO_3^-$ influx declined from 9.41 to 3.63 and 1.69 μmol g^{-1} h^{-1}, respectively (Figs. 1 and 5). Likewise, K_m values for $^{13}NO_3^-$ influx declined from 79 to 45 and 30 mmol m^{-3}, respectively, as root $[NO_3^-]$ increased (Fig. 5).

$^{13}NO_3^-$ influx also obeyed Michaelis-Menten kinetics in uninduced plants. However, a comparison of Figures 1 and 2 reveals that $^{13}NO_3^-$ influx was considerably lower in uninduced plants than in induced plants, and that $^{13}NO_3^-$ influx saturated at a much lower $[NO_3^-]_o$ in uninduced plants than in induced plants. The V_{max} for influx in uninduced plants was calculated to be 0.344 μmol g^{-1} h^{-1} compared to a value of 9.41 μmol g^{-1} h^{-1} in plants induced for 24 h in 0.1 mol m^{-3} $[NO_3^-]_o$. The K_m for $^{13}NO_3^-$ influx in uninduced plants was determined to be 20 mmol m^{-3}. Thus NO_3^- pretreatment initially caused V_{max} and K_m to increase. The initial increase of V_{max} following induction (about 27-fold) was much larger than the increase of K_m (about 4-fold). However, longer pretreatment or pretreatment at higher $[NO_3^-]_o$ caused these kinetic parameters to decrease (Fig. 5).

High-Concentration System

As $[NO_3^-]_o$ was increased beyond the concentration range in which saturation of $^{13}NO_3^-$ influx occurred, a second system for $^{13}NO_3^-$ influx, linearly dependent on $[NO_3^-]_o$, became apparent in both uninduced (Figs. 2 and 4) and

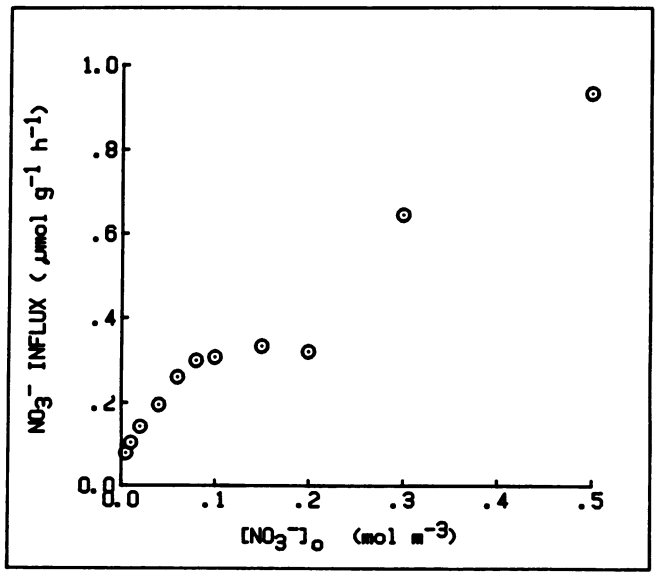

Figure 2. $^{13}NO_3^-$ influx from 0.005 to 0.5 mol m^{-3} $[NO_3^-]_o$ into roots of uninduced plants. (Symbols as in Fig. 1.).

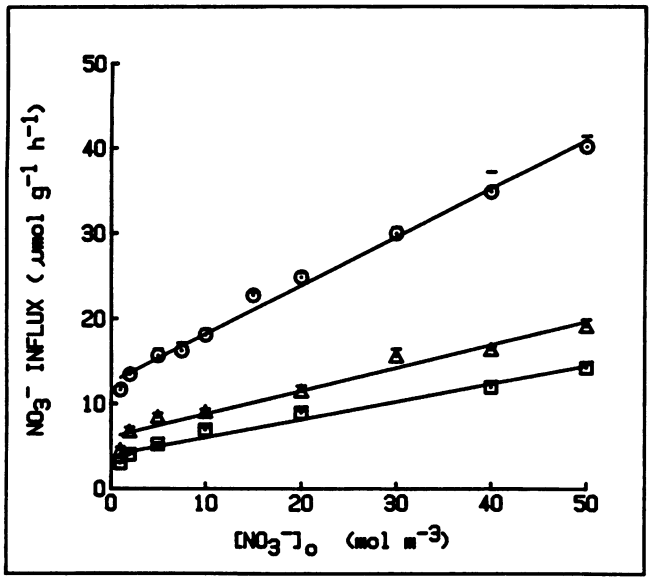

Figure 3. $^{13}NO_3^-$ influx from 1 to 50 mol m^{-3} $[NO_3^-]_o$ into roots of induced plants. (Symbols as in Fig. 1.)

induced (Fig. 3) plants. However, whereas the high-concentration system was apparent at 0.3 mol m^{-3} $[NO_3^-]_o$ in uninduced plants, the same system was not evident in induced plants until $[NO_3^-]_o$ exceeded 1 mol m^{-3}. In plants induced with NO_3^- for 1 d and uninduced plants the rate constants (slopes relating influx to $[NO_3^-]_o$) for these first-order (linear) $^{13}NO_3^-$ influxes were rather similar: 0.57 and 0.64, respectively (Figs. 3 and 4).

Clearly, the observed high concentration fluxes in a biphasic system represent the sum of the two transport systems. Since the high concentration system in induced plants receives a large contribution from the (induced) low concentration system, comparisons of high concentration fluxes in induced and uninduced plants are complicated by this induction effect. Subtraction of the V_{max} of the low-concentration system in induced plants from the observed fluxes in the high concentration range (1–50 mol m^{-3} $[NO_3^-]_o$) of 1-d-induced plants gave influx values which corresponded closely to the influxes in the high concentration range (1–50 mol m^{-3} $[NO_3^-]_o$) of uninduced plants (Fig. 6).

DISCUSSION

The present observations documenting a dual pattern of $^{13}NO_3^-$ influx in barley are consistent with earlier observations of net uptake of NO_3^- by induced barley (21), *Arabidopsis thaliana* (4), and maize (24). The linear kinetics observed at high $[NO_3^-]_o$ confirm the observations of Mellis (21) in barley and Pace and McClure (24) for maize. In the diatom *Skeletonema costatum*, a similar biphasic system for NO_3^- uptake has been reported (28). However, in this organism the linear system became evident at much lower concentrations (10–20 mmol m^{-3}) than was the case for higher plants.

The linear kinetics observed in higher plants are reminiscent of the linear high-concentration K^+ fluxes reported in corn roots by Kochian and Lucas (14). However, the studies by Doddema and Telkamp (4) established that the high-concentration system of wild type and a B25 mutant of *A. thaliana* exhibited saturation kinetics. Only a B1 mutant showed linear kinetics. Such apparently conflicting results have been reported for other ions, for example K^+. While the kinetic data of Kochian and Lucas (14) established linear kinetics for K^+ influx in maize roots, which they interpreted as the result of channel-mediated transport, Epstein and Rains (5) had earlier reported complex multiple saturation curves in barley at high external $[K^+]$. Whether these differences are species-based or due to methodological differences has never been satisfactorily resolved.

The low concentration system for $^{13}NO_3^-$ influx, showing characteristic Michaelis-Menten kinetics and saturation in the concentration range from about 0.2 to 0.5 mmol m^{-3} $[NO_3^-]_o$ are in agreement with earlier published reports for net uptake of NO_3^- or $^{13}NO_3^-$ influx in several plant species (*e.g.* barley: 16, 18, 26; *Arabidopsis*: 4; wheat: 9, corn: 24, and rice: 30). The present report also demonstrates that $^{13}NO_3^-$ influx in the low concentration system is under negative feedback control from tissue NO_3^- and/or some product(s) of NO_3^- assimilation. A plot of V_{max} and K_m against root $[NO_3^-]$ shows that as root $[NO_3^-]$ and/or root N status increased during NO_3^- pretreatment, V_{max} and K_m values declined (Fig. 5). However, in uninduced plants (not shown in Fig. 5), V_{max} and K_m values were 0.344 μmol g^{-1} h^{-1} and 20 mmol m^{-3}, respectively. Had they been included in Figure 5, it would be apparent that, consistent with the results of Lee and Drew (18), induction initially causes increased V_{max} and K_m values. It must be emphasized, however, that the differences in K_m arising from manipulating the N status of barley roots in our study and that of Lee and Drew (18) are rather small compared to the changes of V_{max}. Our results concerning the effects of altered NO_3^- (and/or N) status of barley roots

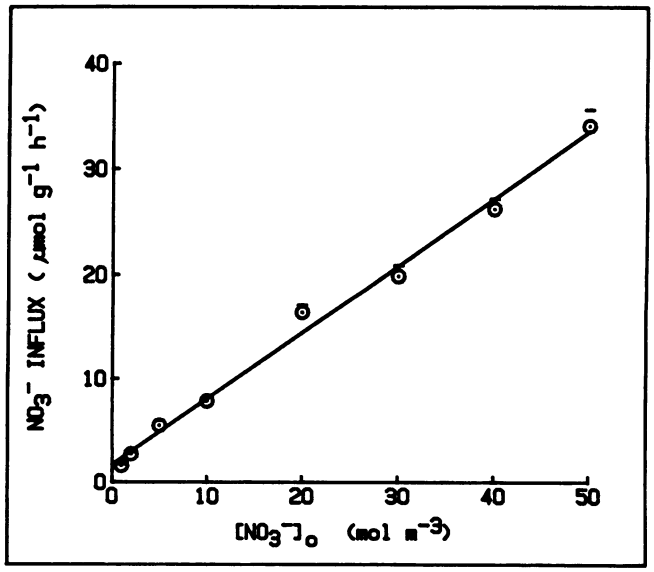

Figure 4. $^{13}NO_3^-$ influx from 1 to 50 mol m^{-3} $[NO_3^-]_o$ into roots of uninduced plants.

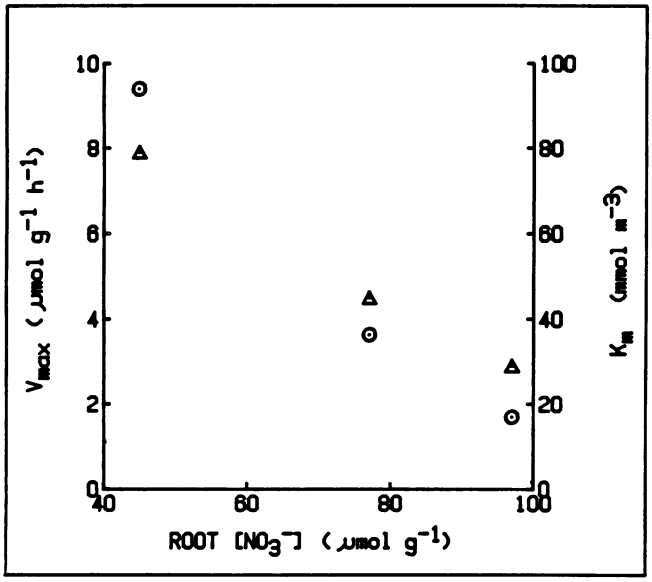

Figure 5. V_{max} (O) and K_m (Δ) for $^{13}NO_3^-$ influx into roots of induced plants in the low-concentration range (0.005–0.5 mol m^{-3} $[NO_3^-]_0$) in relation to root $[NO_3^-]$.

on the kinetic parameters (K_m and V_{max}) for $^{13}NO_3^-$ influx differ from those of Lee and Drew (18) in one regard. These authors observed that lowering root $[NO_3^-]$ increased V_{max} but failed to change K_m. However, in their study root $[NO_3^-]$ was lowered by completely withholding NO_3^- from plants previously grown in 1.5 mol m^{-3} $[NO_3^-]_0$ for 3 d. In our previous studies (29), withholding NO_3^- has had the effect of rapidly lowering influx. It is unclear whether the differences we emphasize here are genotypic in origin or the result of differences in experimental approach.

Our current observations and those of Lee and Drew (18) regarding the changes of $^{13}NO_3^-$ influx associated with increased N status are in conflict with earlier reports suggesting that NO_3^- influx is insensitive to negative feedback control and that net NO_3^- uptake is regulated by alterations of efflux (3, 8). However, we have recently demonstrated (29) that negative feedback effects upon NO_3^- influx are evident only after peak induction has been attained; in fact, during induction NO_3^- influx is positively correlated with root $[NO_3^-]$. Thus, a plot of $^{13}NO_3^-$ influx *versus* root $[NO_3^-]$ takes the form of a parabola and a comparison of fluxes in the two physiological 'states' (induced/feedback) may fortuitously show little differences, thus obscuring the phenomenon of negative feedback inhibition (26). Investigating the influence of NO_3^- status on NO_3^- influx by completely withholding NO_3^- may further complicate the interpretation of results because of the decay of influx ('deinduction') associated with complete withdrawal of NO_3^- (29).

The source of negative feedback effects on $^{13}NO_3^-$ influx associated with altered N status are unclear. Lee and Rudge (19) proposed that negative feedback was mediated by some product(s) of NH_4^+ assimilation rather than by NO_3^- or NH_4^+ *per se*. We have argued, however, that vacuolar $[NO_3^-]$ may exert an effect, albeit indirect, upon negative feedback inhibition of NO_3^- influx through effects on the rate of NO_3^- flow through the GS-GOGAT pathway (29).

It is generally considered that the induction of NO_3^- uptake requires *de novo* protein synthesis (11, 12). Some observations have been interpreted to suggest that the very low rates of NO_3^- influx in the low-concentration range by uninduced plants (sometimes referred to as constitutive uptake) may be due to a transport system which is distinct from that responsible for influx (the inducible system) which develops following exposure to NO_3^- (2). This suggestion is based on the differential effects of puromycin on NO_3^- uptake by uninduced and partially induced plants. These experiments demonstrated that the inducible system was more labile than the constitutive system (2, 12). There is another explanation of the differential effects of puromycin: the concentration of NO_3^- employed in the comparison of induced and uninduced NO_3^- uptake by Jackson *et al.* (12) was 0.5 mol m^{-3}. Our kinetic comparisons reveal that there is a large contribution of the linear high-concentration system (Fig. 2) to influx by uninduced plants at this concentration. By contrast, NO_3^- influx into roots of induced plants via the high-concentration system is not apparent until $[NO_3^-]_0$ exceeds 1 mol m^{-3}. Hence the comparison being made in the puromycin study may have been one of low/high concentration systems rather than induced/uninduced systems. In addition, the difference in K_m for $^{13}NO_3^-$ influx by induced and uninduced plants (18) was taken to suggest that the two systems were distinct. Our results, however, clearly show that among induced plants, K_m varied continuously over a wide range of root $[NO_3^-]$. We are, therefore, not convinced that the low-concentration sys-

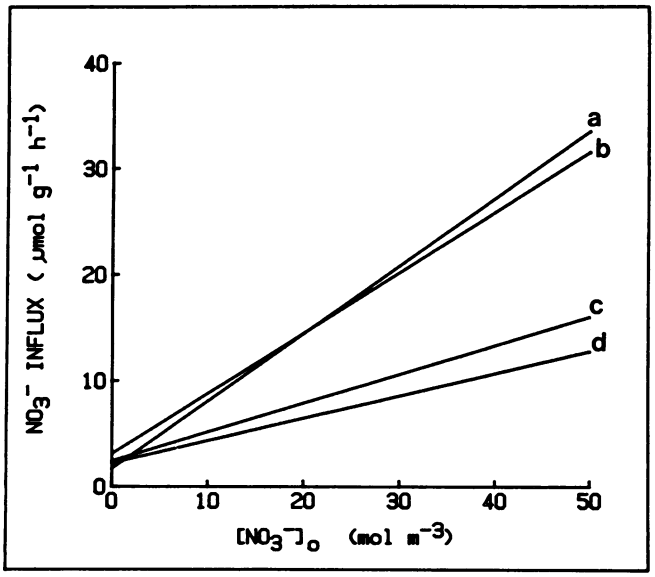

Figure 6. Estimated values for NO_3^- influx into roots of uninduced and induced plants in the high-concentration range obtained by subtracting V_{max} of the low-concentration system from observed fluxes in the range 1 to 50 mol m^{-3} $[NO_3^-]_0$ (see text). Uninduced plants (a), plants pretreated with 0.1 mol m^{-3} NO_3^- for 1 d (b), or for 4 d (c), or with 10 mol m^{-3} NO_3^- for 4 d (d).

tem(s) in induced and uninduced plants are mediated by two distinct populations of transporters.

High Concentration System

Figure 6 demonstrated that subtraction of the V_{max} of the low-concentration system of 1-d-induced plants from the observed fluxes in the high-concentration range (1–50 mol m^{-3}) of the same 1-d-induced plants gave influx values which corresponded closely to the influx values of uninduced plants in the same high-concentration range. We conclude that the high-concentration systems of 1-d-induced plants and uninduced plants are essentially identical and that the transport system is constitutive.

It is clear, however, that the high-concentration system, like the low-concentration system, was subject to negative feedback control (Figs. 3 and 6): with increasing root $[NO_3^-]$ both $^{13}NO_3^-$ influx and the first order rate constant for the linear component decreased. Interestingly, negative feedback inhibition of $^{13}NO_3^-$ influx reduced the rate constant by only about 10% (Fig. 6) as root $[NO_3^-]$ increased from levels which were essentially undetectable in uninduced plants to approximately 40 μmol g^{-1} in plants exposed to NO_3^- for 24 h. Further increases in root $[NO_3^-]$ to about 75 (4-d-induced in 0.1 mol m^{-3} $[NO_3^-]_0$) and about 97 μmol g^{-1} (4-d-induced in 10 mol m^{-3} $[NO_3^-]_0$ were correlated with stronger inhibition of influx; rate constants decreased by 57 and 66%, respectively. It is noteworthy that due to negative feedback effects, even the measured fluxes of 4-d-induced plants (*i.e.* without subtraction of low-concentration system component of the flux) from $[NO_3^-]_0 \geq 20$ mol m^{-3} were less than those of uninduced plants (Fig. 3 and 4). We have earlier demonstrated that influx by the low-concentration inducible system is subject to negative feedback inhibition only after 'peak induction' has been attained (29); in plants exposed to 0.1 mol m^{-3} this was achieved after 1 d. Thus, the low-concentration inducible system and the high-concentration constitutive system showed a remarkable similarity as far as the negative feedback responses of these systems are concerned. Clearly, this is crucial if the two systems are to be integrated to maintain internal $[NO_3^-]$ at some 'prescribed' concentration.

Energetic Considerations

Our experiments were not designed to probe energetic aspects of the two NO_3^- transport systems. Nevertheless some comments on this subject are pertinent. According to universal wisdom, NO_3^- transport at low $[NO_3^-]_0$ is considered to be thermodynamically 'uphill,' mediated by an inducible carrier system. This conclusion derives from considerations of the energetics of transporting any anion (but particularly NO_3^-) across an electrically polarized (negative inside) membrane. In addition, there are the observed effects of metabolic inhibitors on NO_3^- transport (2, 6 for review). The observed saturation kinetics in the low concentration range for $^{13}NO_3^-$ influx are consistent with this model.

However, there is little information in the literature regarding the energetics of the high-concentration system. In the present study, the linear response of influx to $[NO_3^-]_0$ is suggestive of a passive movement down the electrochemical potential gradient ($\Delta\tilde{\mu}_{NO_3^-}$), perhaps *via* some NO_3^--specific channel(s). In the case of K^+ influx, it has been proposed that the linear high-concentration system in corn roots is channel mediated (14, 15). Since the original proposal by Kochian and Lucas, K^+ channels have been demonstrated in diverse plant tissues (10, 13). It is evident, however, that to have a favorable $\Delta\tilde{\mu}_{NO_3^-}$ between the outside and the cytoplasm across a negatively charged plasmalemma, either cytoplasmic $[NO_3^-]$ should be very low or $\Delta\psi$ should have to be appropriately high (*i.e.* positive). In the case of uninduced plants (where mean root $[NO_3^-]$ is hardly detectable), it is not difficult to envisage extremely low cytoplasmic $[NO_3^-]$ and hence a favorable $\Delta\tilde{\mu}_{NO_3^-}$ even from relatively low $[NO_3^-]_0$. For example, from 0.3 mol m^{-3} $[NO_3^-]_0$, where the high-concentration system became evident in uninduced plants (Fig. 2), the Nernst equilibrium potentials for NO_3^- were calculated to be −201, −143, and −86 mV, respectively, at values of 0.1, 1, and 10 mmol m^{-3} cytoplasmic $[NO_3^-]$. Clearly, measured electrical potentials in plants are not far removed from these calculated Nernst potentials. It follows from the above arguments that cytoplasmic $[NO_3^-]$ may well be in the nanomolar range and the concept that NO_3^- uptake is universally uphill may therefore be ill-founded. We suggest that the constitutive high-concentration system is activated only when $\Delta\tilde{\mu}_{NO_3^-}$ is in the appropriate direction.

In the case of induced plants, which have been exposed to NO_3^- for periods up to 24 h or even longer, cytoplasmic $[NO_3^-]$ might be expected to be considerably higher than in uninduced plants. However, the resultant of NO_3^- efflux, NO_3^- reduction, NO_3^- delivery to the stele and NO_3^- loading into the vacuole may serve to maintain cytoplasmic $[NO_3^-]$ at relatively low values. Indeed, judging from literature values for the K_m of the enzyme nitrate reductase, cytoplasmic $[NO_3^-]$ might be anticipated to be in the range from 100 to 300 mmol m^{-3}. Nevertheless, Lee and Clarkson (17) estimated cytoplasmic $[NO_3^-]$ by means of compartmental analysis (using $^{13}NO_3^-$) to be 26 mol m^{-3} in barley roots. Even higher values (50–100 mol m^{-3}) were obtained in maize roots by Presland and McNaughton (25). By contrast, estimates of cytoplasmic $[NO_3^-]$ based upon the rates of reduction of NO_3^- to NO_2^- in the absence of external NO_3^- are as low as 0.015 to 0.14 mol m^{-3} (1, 27, and references therein). In their ^{14}N-NMR studies of barley, maize, and pea roots, Belton *et al.* (1) detected only a single NO_3^- pool, attributable to vacuolar NO_3^-. It was inferred that cytoplasmic $[NO_3^-]$, too low to be detected by their method, was markedly lower than vacuolar $[NO_3^-]$. It should be emphasized that the plants used in Belton *et al.*'s study were cultivated in 5 mol m^{-3} NO_3^- solution. Definitive data on cytoplasmic $[NO_3^-]$ is lacking and is obviously critical for the resolution of this question.

In induced plants the linear high-concentration fluxes became apparent at $[NO_3^-]_0$ above 1.0 mol m^{-3}. Selecting values for cytoplasmic $[NO_3^-]$ of 10, 50, or 100 mmol m^3, it can be calculated that passive flux equilibrium would demand that the Nernst equilibrium potentials be −116, −75, or −50 mV, respectively, for the above values of cytoplasmic $[NO_3^-]$ and external $[NO_3^-]$ of 1.0 mol m^{-3}. Hence, if cytoplasmic $[NO_3^-]$ were micromolar rather than millimolar, NO_3^- influx might

be downhill under these conditions. If cytoplasmic $[NO_3^-]$ were 26 mol m^{-3} as proposed by Lee and Clarkson (17) then downhill NO_3^- uptake from 1 mol m^{-3} $[NO_3^-]_0$ would demand an electrical potential difference of +82 mV. The higher estimates of cytoplasmic $[NO_3^-]$ ($\geq$26 mol m^{-3}) are clearly inconsistent with the idea of passive, channel-mediated influx in the high concentration range. On the other hand, if these fluxes are indeed active, it would be necessary to propose a high-concentration transport system with an extraordinarily high K_m value in order to account for the observed linear kinetics.

It is interesting that the high-concentration system for K^+ uptake in corn roots (14) gave no evidence of negative feedback effects from root $[K^+]$ upon K^+ influx; nor was there any difference between low-K^+ and high-K^+ plants with respect to the $[K^+]_0$ at which the high-concentration system became evident. It is known that cytoplasmic $[K^+]$ is maintained at a relatively constant level, independently of K^+ supply (7 for review). Thus, in the case of K^+, the direction of $\Delta\tilde{\mu}_{K^+}$ between the exterior and the cytoplasm should be the same in low- and high-K^+ plants, unless any difference in membrane potential associated with these conditions is large enough to influence $\Delta\tilde{\mu}_{K^+}$. It is evident that a definitive analysis of the energetic basis of the high concentration NO_3^- fluxes will not be possible until precise estimates of cytoplasmic $[NO_3^-]$ become available. Work is presently underway to evaluate the energy dependence of the high concentration fluxes by means of metabolic inhibitors.

CONCLUSIONS

1. NO_3^- influx into roots of induced and uninduced barley plants is biphasic, mediated by a low-concentration saturable system and a high-concentration nonsaturable system. In uninduced plants the high-concentration system takes effect at a lower $[NO_3^-]_0$ (0.2–0.3 mol m^{-3}) than in induced plants (about 1 mol m^{-3}). We propose that this difference is due to the lower $[NO_3^-]_0$ at which $\Delta\tilde{\mu}_{NO_3^-}$ becomes appropriate for downhill transport in uninduced plants.

2. The low-concentration system is inducible by NO_3^- pretreatment and is subject to negative feedback inhibition: pretreatment with NO_3^- for 24 h increased root $[NO_3^-]$, V_{max} and K_m. By contrast, longer pretreatments with NO_3^- increased root $[NO_3^-]$ but decreased V_{max} and K_m.

3. We are not yet convinced that the low-concentration systems in induced and uninduced plants represent two distinct transporter systems (see 2). We have offered an alternate explanation for what appears to be the greater lability of the NO_3^- uptake system in induced (compared to uninduced) plants (11). The low level of NO_3^- uptake in uninduced plants may represent a constitutive (low) level of expression of the same gene(s) which are induced by exposure to NO_3^-. This constitutive level may represent a genetic 'leak,' ensuring that sufficient NO_3^- will enter the tissues when uninduced plants are exposed to NO_3^- to bring about the induction of enhanced NO_3^- uptake.

4. The high-concentration, nonsaturable system is constitutive and not enhanced by exposure to $[NO_3^-]_0$. It may mediate a passive uptake of NO_3^- through NO_3^- specific channels, along the gradient of $\Delta\tilde{\mu}_{NO_3^-}$. Clearly, when uninduced plants are exposed to high concentrations of $[NO_3^-]_0$, induction should occur rapidly so that as the source of $[NO_3^-]_0$ declines, the low concentration system will be available to maximize absorption of NO_3^-.

5. The high-concentration system is subject to negative feedback inhibition only after prolonged exposures to NO_3^-, as in the case of the low-concentration system.

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