The Uptake of NO$_3^-$, NO$_2^-$, and NH$_4^+$ by Intact Wheat (Triticum aestivum) Seedlings

I. INDUCTION AND KINETICS OF TRANSPORT SYSTEMS

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ABSTRACT

The inducibility and kinetics of the NO$_3^-$, NO$_2^-$, and NH$_4^+$ transporters in roots of wheat seedlings (Triticum aestivum cv Yecora Rojo) were characterized using precise methods approaching constant analysis of the substrate solutions. A microcomputer-controlled automated high performance liquid chromatography system was used to determine the depletion of each N species (initially at 1 millimolar) from complete nutrient solutions. Uptake rate analyses were performed using computerized curve-fitting techniques. More precise estimates were obtained for the time required for and the extent of the induction of each transporter. Up to 10 and 6 hours, respectively, were required to achieve apparent full induction of the NO$_3^-$ and NO$_2^-$ transporters. Evidence for substrate inducibility of the NH$_4^+$ transporters requiring 5 hours is presented. The transport of NO$_2^-$ was mediated by a dual system (or dual phases), whereas only single systems were found for transport of NO$_3^-$ and NH$_4^+$. The $K_m$ values for NO$_3^-$, NO$_2^-$, and NH$_4^+$ were, respectively, 0.027, 0.054, and 0.05 millimolar. The $K_m$ for mechanism II of NO$_2^-$ transport could not be defined in this study as it exhibited only apparent first order kinetics up to 1 millimolar.

Conclusions about the induction periods have often been based on accumulative uptake as a function of time. This method does not offer the degree of resolution required to precisely define the extent of induction or the time required for full induction. Small changes in uptake rates are masked in such plots. A rate analysis as a function of time allows a higher resolution of the induction of a transporter. This requires a sensitive and precise analytical method to establish rates over small changes in concentration of the ambient solution. A microcomputer-controlled, highly precise and fully automated HPLC system was used to assay NO$_3^-$, NO$_2^-$, and NH$_4^+$ in this study. A rate analysis of uptake was done using computerized statistical procedures of curve fitting (12).

Little information is available on the uptake kinetics of NO$_3^-$ and the published interpretations for kinetics of NO$_3^-$ and NH$_4^+$ lack unanimity. For example, NO$_3^-$ uptake has been described as resembling a single (19, 29), dual (10, 23), single multiphasic (5) isotherm or the additive result of one saturable plus one nonsaturable, linear component (14). Similarly, NH$_4^+$ transport was mediated by a single (2, 19, 28, 30), two different (3), and a single multiphasic transport system (18). $K_m$ values (Michaelis constant) for transport of these ions also differ substantially: a range of 0.001 to 7.5 mm for NO$_3^-$ and 0.007 to 3.0 mm for NH$_4^+$ have been published previously.

The objective of this study was to characterize the induction and kinetics of the NO$_3^-$, NO$_2^-$, and NH$_4^+$ transport systems in wheat roots.

MATERIALS AND METHODS

Plant Material. Seeds of wheat (Triticum aestivum cv Yecora Rojo) were soaked in aerated deionized H$_2$O at 25°C in darkness. After 24 h the seeds were rinsed several times with deionized H$_2$O, and germinated hydroponically in 0.5 mm CaSO$_4$ by the method described by Rao and Rains (23). On the 8th d, the seedlings were transferred to a growth chamber with a 25/15°C and 16 h/8 h day-night cycle, 550 μE/m$^2$·s quantum flux density, and 65% RH for 2 d. The culture medium on the 8th d was replaced with one-quarter strength Hoagland (13) solution lacking N. Pretreatments, where specified, were started on the 10th d. For preinduction, the seedlings were incubated in 1 mm substrate solution for 24 h. On the 10th d the seedlings were transferred to the growth chamber described below, with the same photoperiod and temperature conditions. The mother seed was still attached to the seedlings and no symptoms of N deficiency were seen.

Experimental Procedure. The experiments were conducted in a growth chamber (25°C, 700 μE/m$^2$·s, 60% RH) which was part of a fully automatic, microcomputer-controlled system consisting of...
of an Apple II Plus (Apple Computers, Cupertino, CA 95014) microcomputer, a HPLC with UV (Holochrome, Gilson Medical Electronics, Inc., Middletown, WI 53562) and fluorescence detectors (Fluorichrome, Varian, Palo Alto, CA 94303), an automatic sampler/injector (12), and a multi-position valve (model A-16, Valco Instrument Co., Inc., Houston, TX 77255). The system was designed to draw samples automatically from the substrate solution at desired time intervals, inject the samples into the HPLC, collect the resulting analytical data, integrate the peaks, and record the concentrations on a printer. This enabled us to monitor the solution concentrations at real time. The system has been detailed elsewhere (12).

Experiments were initiated by placing 8 to 10, 10-d-old seedlings (grown as above) in a Pyrex culture tube (25 mm × 150 mm), containing 50 ml of appropriate substrate solutions. Prior to the transfer, roots were thoroughly rinsed with one-quarter strength Hoagland solution lacking N (or the pretreatment solution) and were held in air for 1 min to drain excessive solution (roots were never blotted or touched). To allow proper mixing of rinse solution adhering to roots and the substrate solution, the first sample was drawn 2 min after the transfer of seedlings. Solutions were continuously aerated to maintain sufficient O₂ and to provide solution mixing. In most experiments, a total depletion of N was allowed to study the relationship between substrate concentration and the uptake rate over the range of 0 to 1 mM. Preliminary experiments showed that the seedlings were capable of reacting to sudden decreases in substrate concentrations by quickly achieving a lower and steady uptake rate within 2 to 4 min. In some experiments, the substrate concentration was held within a narrow range by periodically adding very small aliquots of 1 M solutions to replenish the substrate medium. Sufficient time was allowed for thorough mixing of the added N before the next sample was drawn. Each treatment was replicated at least twice and all experiments were repeated at least thrice. The results reported are from one representative replication. The cited Kᵣ and Vₘₐᵥ values were calculated from the data reported in this paper and generally varied by ±50% and ±10%, respectively, between experiments.

Measurement and Computation of Uptake Rates. The uptake rates of NO₃⁻, NO₂⁻, and NH₄⁺ were measured as the amounts dissolved from 100-μl substrate solutions per unit time. The rates expressed as μmol (g fresh weight-h)⁻¹ were computed using a microcomputer (Apple II Plus) from the concentrations and volumes at consecutive sampling times, which corrected for evapotranspiration and loss of N through sampling.

Since the uptake rates were computed over relatively short time intervals and over small concentration changes, small variations in concentration values could lead to large differences in uptake rates. Therefore, to reduce the effect of random variation, the concentration data were fitted to various equations (from linear to 6th degree polynomial) by using an Apple II Plus microcomputer and a “curve fitter” program (IMI, College Park, PA 16804). The best fit equation was selected on the basis of statistical parameters that indicated the goodness of fit. The significance of fitted curves and the improvement in regression fit contributed by “Extra Sums of Squares” due to each increase in degree of polynomial were tested by the F method of Steele and Torrie (25) and Neter and Wasserman (21), respectively. All fitted curves were highly significant at P < 0.001. A new set of concentration values was then determined from the best fit curves. Usually, the observed and evaluated concentrations were very close; the difference being only a few μM. The reported uptake rates were computed from the best fit curves. For a detailed discussion of this technique see Goyal and Huffaker (12). The concept of fitting curves to such experimental data has been used earlier also (8). In experiments where the ambient concentration was held within a narrow range by periodic additions, the uptake rates were calculated directly from the observed decreasing concentrations.

Analytical Methods. The HPLC method of Thayer and Huffaker (26) was used to assay NO₃⁻ and NO₂⁻. Ammonium in solutions was determined fluorometrically after derivatization with O-phthaldialdehyde in basic medium (12).

Substrate Solutions. One-quarter strength Hoagland solution (13), in combination with various N treatments, was uniformly used. To minimize the drop in pH, 1 mM CaCO₃ was included in solutions containing NH₄⁺. Solution pH was adjusted to 6.5 prior to the start of the experiment. The respective sources of NO₃⁻, NO₂⁻, and NH₄⁺ were KNO₃, KNO₂, and (NH₄)₂SO₄.

RESULTS

NO₃⁻ Uptake. When seedlings were exposed to a 1 mM NO₃⁻ solution, the rate of NO₃⁻ uptake was initially very slow and, after 1.5 h, increased steadily up to 7 h (Fig. 1a). The rate increased despite a progressive decrease in NO₃⁻ concentration that commenced at about 2.5 h. The loss of water through evapotranspiration concentrated the solutions and balanced the very low uptake during the first 2.5 h such that little change in NO₃⁻ concentration occurred during this time. When the ambient concentration was maintained within a narrow range at 1.05 to 0.8 mM the NO₃⁻ uptake rate began to level off after 8 h, and may have required as much as 10 h for full induction of the transporter (Fig. 1b).

When the NO₃⁻ transporter was preinduced, the NO₃⁻ concentration decreased rapidly throughout the course of the experiment without a lag (Fig. 1c). The NO₃⁻ uptake rate decreased with decreasing NO₃⁻ concentration, except during an intermediate concentration range of 0.70 to 0.20 mM when the uptake rate remained quite constant (Fig. 1, c and d). Only one Kₘ (0.027 mM) and one Vₘₐᵥ value (2.6 μmol (g fresh weight-h)⁻¹) were calculated as the NO₃⁻ uptake rate in concentrations higher than 0.70 mM did not achieve apparent zero order up to 1 mM (Fig. 1d).

NO₂⁻ Uptake. The depletion of NO₂⁻ and the resulting uptake rates by seedlings from a 1 mM solution are shown in Figure 2a. Little change in NO₂⁻ concentration occurred during the first 2 h, but after a lag of about 0.5 h, the uptake rate increased steadily up to 6 h (Fig. 2a). When the concentration was maintained relatively constant the NO₂⁻ uptake rate plateaued after 6 h (Fig. 2b).

When the transporter was preinduced, the NO₂⁻ concentration decreased steadily without a lag. The uptake rate achieved an apparent steady state without any time delay, and remained constant for 5 h (Fig. 2c). When the ambient NO₂⁻ concentration decreased below 0.25 mM, the uptake rate steadily decreased (Fig. 2, c and d). A similar response was noted in Figure 2a. A Kₘ of 0.054 mM and a Vₘₐᵥ of 2.0 μmol (g fresh weight-h)⁻¹ were calculated for NO₂⁻ transport.

NH₄⁺ Uptake. When seedlings were placed in a 1 mM NH₄⁺ solution, the concentration decreased steadily without a time lag (Fig. 3a). An NH₄⁺ uptake rate of 1.25 μmol (g fresh weight-h)⁻¹ was recorded for the first 0.5 h. The rate increased until about 5 h, while the NH₄⁺ concentration decreased progressively. The rate of NH₄⁺ uptake decreased rapidly after 5 h when the substrate concentration decreased beyond 1 mM (Fig. 3a).

When the same experiment was conducted with shorter sampling intervals and with relatively constant NH₄⁺ concentration, the results were somewhat different (Fig. 3b). The rate decreased rapidly during the 1st h and briefly plateaued at a lower rate of about 2 μmol (g fresh weight-h)⁻¹ (Fig. 3b). Then, the NH₄⁺ uptake rate increased again and reached a steady state of about 4 μmol (g fresh weight-h)⁻¹ after about 5 h. The trend was reproducible between replications and experiments.

The NH₄⁺ uptake rate of seedlings, preinduced in 1 mM
**NO$_3^-$, NO$_2^-$, and NH$_4^+$ UPTAKE**

**DISCUSSION**

**NO$_3^-$ Uptake.** An induction phase for NO$_3^-$ transport, first shown by Jackson *et al.* (16), has now been established for many plant species. The time required for induction of the transporter varied. Periods of 1 to 2 h for excised corn roots (22), 4 h for wheat seedlings (16), 6 to 7 h for dwarf bean seedlings (5), at least 8 and possibly 10 h for wheat seedlings (this study) have been recorded. The reasons for the differences may be partially attributed to the methodology employed. When evaluations of the amount of induction and time required for induction are based on a curve of accumulative uptake versus time, both parameters are underestimated. Such plots do not have the sensitivity to depict small changes in uptake rates. To substantiate this point, the data in Figure 1a were plotted as accumulative uptake versus time (Fig. 1e). Uptake of NO$_3^-$ seems to have achieved linearity after 5 h in Figure 1e; whereas, the rate actually increased up to 7 h in a solution allowed to deplete (Fig. 1a). During these 2 h, the rate increased from 2.2 $\mu$mol (g fresh weight-h)$^{-1}$ at 5 h to 3.1 $\mu$mol (g fresh weight-h)$^{-1}$ at 7 h; ~ a 40% increase (Fig. 1a). Full induction of the NO$_3^-$ transporter may have required up to 10 h at 1 mM when the concentration did not limit the induction and/or activity (Fig. 1b). The scatter of the data in Figure 1b was largely due to the concentration dependency of activity of the NO$_3^-$ transporter in the range used, 0.8 to 1.00 mM. In contrast, the scatter in similar experiments with NO$_2^-$ and NH$_4^+$ was much less (Figs. 2b and 3b) because the activity of these transporters was independent of substrate concentration in the range of about 0.25 to 1.00 mM (Figs. 2d and 3d). The best-fit curves to the data from several experiments suggested that the full induction of the NO$_3^-$ transporter may have required 10 ± 0.5 h.

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**FIG. 1.** (a and b) Nitrate uptake by wheat seedlings as a function of time. Seedlings were placed in 1.0 mM NO$_3^-$ at time zero. a, The concentration data points (□) represent actual observed values. The smooth line through the concentration points is the best-fit curve. The uptake rates (■) were computed from the best-fit curve. b, The concentration was adjusted by adding NO$_3^-$ at times indicated by arrows. The concentration data points (□) represent actual observed values. The uptake rates (■) were computed from observed concentration values. The smooth line through the uptake rate points is the best-fit curve. c-e) Nitrate uptake by wheat seedlings pre-incubated in 1.0 mM NO$_3^-$ for 24 h. c, The concentration data points (□) represent actual observed values. The smooth line through the concentration points is the best-fit curve. The uptake rates (■) were computed from the best-fit curve. (c-e) Nitrate uptake rate data of Figure 1c plotted as a function of NO$_3^-$ concentration. The rates were plotted against the mean of every two consecutive concentration data points. e, The NO$_3^-$ uptake rate data of Figure 1a plotted as accumulative NO$_3^-$ uptake.
FIG. 2. (a and b) Nitrite uptake by wheat seedlings as a function of time (□, concentration; ■, rate). a, All other conditions were the same as in Figure 1a; b, all other conditions were the same as in Figure 1b. (c and d) Nitrite uptake by wheat seedlings preincubated in 1.0 mM NO₂⁻ for 24 h (□, concentration; ■, rate). c, All other conditions were the same as in Figure 1c; d, the NO₂⁻ uptake rate data of Figure 2c plotted as a function of concentration. The rates were plotted against the mean of every two consecutive concentration data points.

FIG. 3. (a and b) Ammonium uptake by wheat seedlings (□, concentration; ■, rate) as a function of time. a, All other conditions were the same as in Figure 1a; b, all other conditions were the same as in Figure 1b. (c and d) Ammonium uptake by wheat seedlings preincubated in 1.0 mM NH₄⁺ solution for 24 h (□, concentration; ■, rate). c, All other conditions were the same as in Figure 1c; d, the NH₄⁺ uptake rate data of Figure 3c plotted as a function of concentration. The rates were plotted against the means of every two consecutive concentration data points.
The net uptake rate of NO$_3^-$ by plant roots during the induction period could be considered a summation of two vectors acting in opposite directions: (a) induction of transporter—this would increase the uptake rate with time until a steady-state is reached; and (b) ambient NO$_3^-$ concentration—a progressive depletion of concentration would result in lower uptake rates. Concentration dependence of NO$_3^-$ uptake, except in the range of 0.25 to 0.5 mM, has been shown (23; Fig. 1d). Therefore, the increasing uptake rate with decreasing concentration (Fig. 1a) shows that the increase in uptake rate caused by induction of the NO$_3^-$ transporter was greater than the decrease in uptake rate resulting from the decrease in NO$_3^-$ concentration. Hence, it is possible to observe an apparent constant uptake rate due to decrease in concentration, even though the transporter has not been fully induced.

The effects of factors such as acclimation, negative feedback, diurnal variations were minimal in this study, since NO$_3^-$ uptake, after induction, is linear up to 24 h in barley (1) and wheat seedlings (SS Goyal, RC Hufnäker, unpublished data) from a 1 mM solution of NO$_3^-$. If the above factors played a significant role, linearity of uptake would not occur.

It is unlikely that any increase in growth rate of seedlings due to provisions of N contributed significantly to the observed induction of the NO$_3^-$ transporter. The seedlings used in the study still had the mother seed attached and showed no symptoms of N deficiency. Furthermore, the induction pattern of NO$_3^-$ uptake by wheat seedlings pretreated with NH$_4^+$ was similar to those receiving no external N (27).

The induction of the NO$_3^-$ transporter in excised corn roots was concentration dependent (22). In contrast, Breteleer and Nissen (5) showed the induction of the NO$_3^-$ transporter in Dwarf beans was independent of substrate concentration. In our study, the rate of induction began to level off after 6 h in a depleting solution (Fig. 1a), at about 0.6 mM. When the concentration was maintained relatively constant at about 1.0 mM, a linear increase in induction was recorded up to about 8 h, and 10 h was required for full induction (Fig. 1b). The activity of the NO$_3^-$ transporter remained constant in the concentration range of about 0.7 to 0.2 mM (Fig. 1d; 23). Had the NO$_3^-$ concentration not limited the amount of induction in Figure 1a, further induction should have been detected up to 8 h or until the NO$_3^-$ concentration limited the transporter activity (below 0.2 mM). This suggests that, in depleting solutions, the induction may be affected by the increase in concentration.

Concentration Dependency. The concentration-dependent NO$_3^-$ uptake in higher plants is currently believed to occur via one of four ways: (a) two different uptake systems or carriers—one mediating uptake at lower concentrations and the other at higher concentrations (10, 23); (b) a single uptake system (19, 29); (c) a single uptake system with distinct concentration-dependent phases (5); (d) a single saturable carrier-mediated uptake plus a simple diffusion component (14). The NO$_3^-$ transport pattern observed in this study fits either the “dual-mechanism” (11) or the multiphasic concept (5) of transport; however, the multiphasic concept has recently been criticized (4). Mechanism I (operative at lower concentrations) achieved a maximal velocity at a NO$_3^-$ concentration of about 0.2 mM. Mechanism II exhibited only apparent first order kinetics up to 1 mM. Independence of NO$_3^-$ uptake rate from substrate concentration in the range of 0.2 to 0.7 mM, repudiates the possibility of a simple diffusion component.

The V$_{max}$ and K$_m$ for mechanism I were respectively, 2.6 μmol (g fresh weight-h)$^{-1}$ and 0.027 mM. This is in general agreement with a K$_m$ of 0.023 mM for maize (29), 0.033 mM for perennial rye-grass (19), 0.040 mM for mechanism I in Arabidopsis (10), and 0.001 to 0.025 mM for barley and 0.005 to 0.015 mM for beans (5). However, K$_m$ values higher than found in this study have also been reported; 0.11 mM for mechanism I in barley (23), 0.25 mM for barley (7), and 7.5 mM for excised corn roots (22). Explanations for variations in K$_m$ values such as multiple kinetic phases of NO$_3^-$ uptake, variations in endogenous NO$_3^-$ levels of plants (5), differential molybdenum status of roots (6), and intensive breeding of some species for higher nitrogen response (23) have been proposed. Additionally, since NO$_3^-$ efflux by roots is affected by the NO$_3^-$ content of roots (5, 9), factors affecting NO$_3^-$ reduction during the plant growth may also affect net NO$_3^-$ uptake rate and its K$_m$. A NO$_3^-$ uptake system positively correlated with nitrate reductase activity has also been proposed (6, 24). Moreover, since the NO$_3^-$ transport system requires a longer time for full induction than previously believed (this study), some studies may have lacked steady-state conditions.

NO$_3^-$ Uptake. The induction of the NO$_3^-$ transporter required about 6 h (Fig. 2a, and b). An induction period of about 4 h for the NO$_3^-$ uptake system (in 0.5 mM NO$_3^-$) in wheat seedlings has been reported previously (17).

The kinetics of NO$_3^-$ uptake by wheat seedlings (preincubated in 1 mM NO$_3^-$ for 24 h) followed a hyperbolic trend resembling a Langmuir isotherm over the range of 0 to 1 mM (Fig. 2c). Thus, it seems that NO$_3^-$ uptake by wheat seedlings is mediated by a single uptake system. In contrast, NO$_3^-$ uptake by barley seedlings over the range of 0.5 to 6 mM has been described as the additive result of a linear simple diffusion and a hyperbolic saturation transfer (15). A small drop in NO$_3^-$ uptake rate was consistently observed at about 0.6 mM NO$_3^-$ concentration which is not understood at this time.

NH$_4^+$ Uptake. In this study, an initial higher rate of NH$_4^+$ disappearance from the uptake solutions was observed. This agrees well with previous reports (20) and may represent the filling of the Apparent Free Space (“outer” space + cation exchange sites) (20). In contrast with previous studies, the high initial rate in our study was never constant and decreased rapidly with time until about 1 h. Moreover, this phenomenon was not observed with the anions, NO$_3^-$ and NO$_2^-$ (Figs. 1b and 2b). The decreasing rate of NH$_4^+$ disappearance during the 1st h could be due to the equilibration of at least four factors: cation exchange capacity of roots, filling of “Outer Space,” a possible unknown amount of constitutive transporter, and induction of the transporter. Our unpublished results show that a new steady state rate of NH$_4^+$ uptake is established in 2 to 4 min after seedlings are placed in a saturated solution of NH$_4^+$ (either step-up or step-down). Thus the outer space equilibrates rapidly. During the 1st h the active transport activity was insufficient to produce a net positive increase in rate; hence, NH$_4^+$ exchange on the root exchange sites, remained as a possible main effector producing a constantly decreasing rate during that time.

The induction of the NH$_4^+$ transporter became apparent after the “outer” space and cation exchange sites were saturated. Then the NH$_4^+$ uptake rate increased (Fig. 3, a and b). The induction required 5 h as shown from depletion of the substrate solution (Fig. 3a) and from results where the substrate concentration was held relatively constant (Fig. 3b). The initial rapid uptake phase, lack of a very precise NH$_4^+$ assay method, and longer time intervals between data points on time course curves, possibly masked the detection of an inducible NH$_4^+$ transporter in previous studies. It is also conceivable that little uptake of NH$_4^+$ may have occurred actively at time zero and that it increased as the transporter was induced. However, from these data it is difficult to determine the actual course of the induction curve during the first 1 h.

Published reports show that NH$_4^+$ uptake by plant roots is mediated by a single (2, 19, 28, 30), or two different (3), or a single multiphasic mechanism (18). In the present study, NH$_4^+$ uptake kinetics resembled a single Langmuir isotherm in a
concentration range of 0 to 1 mm with saturation achieved at about 0.3 mm (Fig. 3d).

As compared to a Km of 0.05 mm found in the study for the NH₄⁺ transporter, values of 0.11 mm (30) and 0.013 mm (2) for maize, 0.4 mm for perennial rye-grass (19), 0.007 mm for wheat (28), 0.2 (Km), and 3.0 mm (Km) for excised maize roots (3) have been reported.

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