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Nickel in Plants

I. UPTAKE KINETICS USING INTACT SOYBEAN SEEDLINGS[1]

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ABSTRACT

The absorption of Ni^{2+} by 21-day-old soybean plants (*Glycine max* cv. Williams) was investigated with respect to its concentration dependence, transport kinetics, and interactions with various nutrient cations. Nickel absorption, measured as a function of concentration (0.02 to 100 μM), demonstrated the presence of multiple absorption isotherms. Each of the three isotherms conforms to Michaelis-Menten kinetics; kinetic constants are reported for uptake by the intact plant and for transfer from root to shoot tissues. The absorption of Ni^{2+} by the intact plant and its transfer from root to shoot were inhibited by the presence of Cu^{2+}, Zn^{2+}, Fe^{2+}, and Co^{2+}. Competition kinetic studies showed Cu^{2+} and Zn^{2+} to inhibit Ni^{2+} absorption competitively, suggesting that Ni^{2+}, Cu^{2+}, and Zn^{2+} are absorbed using the same carrier site. Calculated K_m and K_i constants for Ni^{2+} in the presence and absence of Cu^{2+} were 6.1 and 9.2 μM, respectively, whereas K_m and K_i constants were calculated to be 6.7 and 24.4 μM, respectively, for Ni^{2+} in the presence and absence of Zn^{2+}. The mechanism of inhibition of Ni^{2+} in the presence of Fe^{2+} and Co^{2+} was not resolved by classical kinetic relationships.

Increasing development of fossil fuel resources, application of sludges to agricultural lands, and continuing release of industrial wastes will undoubtedly result in a redistribution of trace metals in the environment. These anthropogenic releases can be expected to increase soil levels of trace elements such as Ni^{2+}, resulting in a concomitant increase in the concentration of Ni^{2+} in plants and possibly in the food chain. Although Ni is considered a nonessential element in the nutrition of both plants and animals, it is present in substantial quantity in all living organisms (1). Plants have been shown to accumulate Ni^{2+} in both vegetative tissues (10) and seeds (13), and therefore represent a source of Ni^{2+} to primary and secondary consumers and ultimately man.

A majority of research on trace element pollutants has been concerned with effects on growth and metabolism (10, 13), and plant availability from sludge-amended soils (6). Few studies have dealt with defining the physiological competence of the absorption system in plants for nonessential trace elements and their interaction with nutrient ions. Cutler and Rains (7) showed that the apparent uptake of Cd^{2+} by intact barley was nonmetabolic with accumulation being a function of physical adsorption and diffusion. No information of this type is available for Ni^{2+} and few physiological data are available for other nonessential trace elements in higher plants.

The present study was initiated to characterize the absorption of Ni^{2+} by intact soybean plants. Relatively short term experiments were employed to define the uptake kinetics of Ni^{2+}, and to elucidate the relationship of Ni^{2+} transport to that of nutrient ions.

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MATERIALS AND METHODS

Plant Culture. Seeds of *Glycine max* cv. Williams were germinated on moist filter paper and individual seedlings transferred to 600-ml beakers containing 500 ml of aerated nutrient solution 3 days following germination. The nutrient solution contained 150 mg of KCl, 120 mg of $MgSO_4$, 946 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 68 mg of KH_2PO_4, 0.06 mg of $ZnSO_4 \cdot 7H_2O$, 0.69 mg of H_3BO_3, 0.017 mg of $CuCl_2 \cdot 2H_2O$, 0.024 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.022 mg of $MnCl_2 \cdot 4H_2O$, and 0.60 mg of Fe^{+3} (as Fe EDDHA) per liter. The pH was adjusted to 5.8 and solutions changed three times a week. Plants were maintained in controlled-environment chambers with a 16/8 hr light cycle (~500 $\mu E\ m^{-2}\ sec^{-1}$, PAR, at leaf surface), a day/night temperature cycle of 26/22 C, and 50% RH.

Nickel Uptake. Evaluation of Ni transport was performed using 21-day-old plants. Prior to use, plants were transferred from nutrient solution to 0.5 mM $CaCl_2$ solution (pH 5.8) for 2 hr to allow for desorption of possible competing ions from root surfaces. Individual plants were subsequently transferred to 0.5 mM $CaCl_2$ solutions (500 ml) containing 0.01 to 200 μM $NiCl_2$ traced with ^{63}Ni (0.69 μCi $^{63}Ni^{2+}/\mu$mol Ni^{2+}), pH 5.8. Following a 2-hr uptake period, roots were gently blotted and placed in 0.5 mM $CaCl_2$ solution containing 10 to 100 μM Ni^{2+} to desorb adsorbed Ni^{2+} traced with $^{63}Ni^{2+}$. Sorption of Ni^{2+} to glassware was not of significant magnitude to affect solution concentration. Shoot and root tissues were analyzed for $^{63}Ni^{2+}$ content separately. Tissues were digested with 10 M HNO_3, clarified with H_2O_2, and $^{63}Ni^{2+}$ activity determined by liquid scintillation spectrometry, with appropriate quench correction. Uptake rates for Ni^{2+} were expressed on the basis of root dry weight, which included only lateral roots; fibrous roots were included with the shoot for $^{63}Ni^{2+}$ analysis.

Ion competition studies were performed using the above protocol. Plants were allowed to accumulate Ni^{2+} for 2 hr from 1.0 to 5.0 μM $NiCl_2$ solutions in the absence and presence of specific concentrations of Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+}. Interpretation of results is based on multiphasic uptake mechanisms with quantitation of kinetic constants using Lineweaver-Burk plots.

RESULTS AND DISCUSSION

The use of intact plants to study the uptake behavior of nutrient and nonnutrient elements can effectively augment cellular and tissue studies by providing a quantitative estimate of the potential for root absorption and subsequent transfer to shoots while maintaining the physiological and structural integrity of the system. Use of intact plants for analysis of uptake kinetics is complicated by an inability to quantitate that portion of the root actively involved in transport and resolve active uptake into the symplast

from physical sorption processes in the apoplast. Reasonably consistent absorption data were obtained in these studies by employing soybean seedlings of similar age and root mass, and by removal of sorbed $^{63}Ni^{2+}$ from root surfaces. While these parameters are important in obtaining reproducible results from large numbers of individual plants used in these studies, the effect of plant age, or more specifically root mass, relates mainly to the need to quantitate absorption based on some measure of absorptive surface. For purposes of these studies, plants from 15 to 21 days of age proved to be most suitable from the standpoint of absorption rate *versus* solution volume (minimum depletion of ions), the relative proportion of roots involved in absorption, and the presence of a developed shoot with two trifoliates.

Figure 1 shows the effect of several desorption solutions (pH 5.8) on the removal of surface sorbed $^{63}Ni^{2+}$. Solutions containing either 0.5 mM $CaCl_2$ or nutrient solution were effective in removing 12% of Ni^{2+} after 30 min, while 21% of Ni^{2+} was desorbed by solutions of 15 μM $NiCl_2$ in 0.5 mM $CaCl_2$. The proportion of Ni^{2+} which was exchangeable or readily diffusible is comparable with desorption data for readily exchangeable nutrilites (2, 8), and substantially less than that reported by Cutler and Rains (7) for Cd^{2+} which shows a marked tendency toward sorption to cell wall material. Use of plants with similar root mass, followed by desorption of exchangeable Ni^{2+}, resulted in reproducible uptake values in subsequent studies.

Preliminary experiments showed Ni uptake from 20 μM solutions by 15-day-old intact plants (2-hr treatment) to be independent of pH from 4.5 to 7.0. The lack of pH effect may be due to the persistence of soluble Ni^{2+} over this pH range with hydrolysis products forming only at pH ~7.0 and above. Uptake rates of Ni^{2+} by 21-day-old plants from 1.0 μM $NiCl_2$ solutions (500 ml) were constant for 4 hr, at which time uptake had reduced the solution concentration by ~10%. Similarly, uptake rates (transfer from root to shoot) for shoots were constant after a 15- to 30-min period. Since reduction of Ni^{2+} concentration below 90% of starting concentrations resulted in a detectable decrease in uptake rate for the intact plant, solution volumes were increased to 1 liter in treatments below 0.2 μM $NiCl_2$ to eliminate significant reductions in solution Ni over the 2-hr uptake periods.

Concentration-dependent Uptake. Multiphasic isotherms are a common characteristic of ion uptake in plants. Such isotherms have been reported for numerous nutrient ions and closely related nonnutrient analogs (9, 11, 12). Little is known concerning the behavior of trace contaminates like Ni^{2+} other than their effects on plant growth and metabolism (10). Nickel concentrations employed in these studies were selected to represent the range of available nickel expected to be encountered in soil solution (0.4–4 μM for endogenous Ni) while maintaining concentrations that would not adversely affect metabolism or absorption processes. In previous studies, *G. max* failed to show any adverse effects of Ni^{2+} on shoot dry matter production or seed yield when grown to maturity on Ritzville silt-loam soil amended with 10 μg/g Ni^{2+} (~250 μM soluble Ni species in soil solution based on extractable Ni), or cultured hydroponically for 30 days in nutrient solutions containing 10 μM $NiCl_2$.

The uptake of Ni^{2+} by intact soybean plants, measured as a function of concentration (0.02–100 μM), results in absorption curves (Fig. 2) characteristic of nutrient ion uptake in higher plants. The absorption isotherms for both the intact plant and the shoots alone exhibit discontinuous transition. Although both sets of data exhibit three phases with similar transition points, there are apparent differences in their saturation kinetics.

Kinetic constants were calculated from Lineweaver-Burk plots for each of the phases (Fig. 2) for both root and shoot transport processes (Table I). A comparison of calculated K_m and V_{max} values for transfer of Ni^{2+} across the outer root membranes and stelar parenchyma cells indicates that there are differences in both the apparent affinity and potential rate of transfer across these membrane barriers. Although the kinetic data for Ni^{2+} transfer to shoots are based on the concentration of Ni^{2+} in the root-bathing solution and not its concentration in the cytoplasm of the stelar parenchyma, it is useful in demonstrating the selectivity of the absorption processes and regulation of transfer from root compartments to shoot. It is apparent from the $^{63}Ni^{2+}$ data (Fig. 2 and Table I) that the rate of accumulation of Ni^{2+} by plants is dependent on its concentration in soil solution, and that the transfer of Ni^{2+} from root to shoot is regulated by the root. In the latter instance, the transfer of Ni^{2+} from root to shoot may be controlled by partitioning of absorbed Ni^{2+} between transport and storage pools, or may be under metabolic regulation by cells of the stelar parenchyma. Whatever the mechanism, the rate of transfer of Ni^{2+} from roots to shoots is only 3 to 9% of the rate for root absorption from the external solution over the course of the experiment.

Interaction of Ni^{2+} with Nutrient Ions. Nickel is not considered to be an essential element in plant nutrition (10), yet its uptake behavior is characteristic of nutrient ions. This suggests that Ni^{2+} may be acting as an analog of an essential species for which effective transport mechanisms are operating. To determine

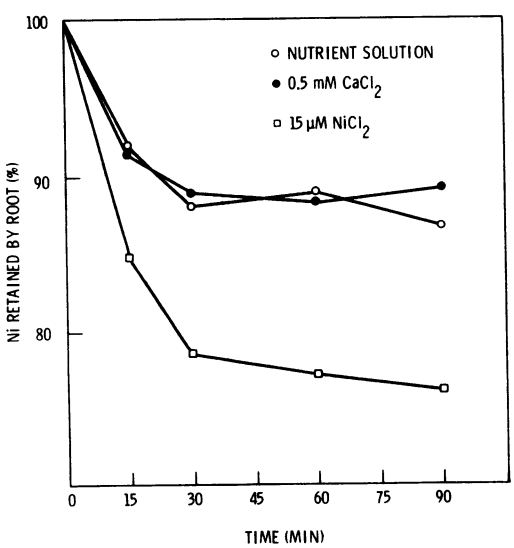

FIG. 1. Desorption of Ni^{2+} from intact roots. Nickel accumulated by 15-day-old soybean plants for 2 hr from 1.0 μM solutions. Efflux solution contained either 0.5 mM $CaCl_2$, nutrient solution, or 15 μM $NiCl_2$.

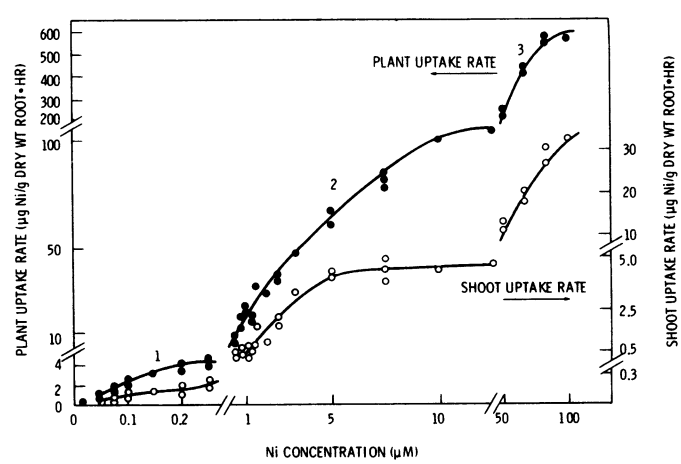

FIG. 2. Effect of nickel concentration on uptake by 21-day-old intact soybeans. Isotherms represent uptake rate for whole plant and rate for transfer from root to shoot.

Table I. Kinetic constants for nickel uptake by roots and shoots of 21-day-old intact soybean plants.

Isotherm (Phase)	Tissue	Concentration Range (μM)	K_m[1] (μM)	V_{max}[2] (μg/g dry wt root·hr)	Coefficient of Determination[3] (r^2)
One	Root + Shoot	0.075 - 0.25	0.51	12.9	0.927
	Shoot	0.075 - 0.25	0.26	0.395	0.713
Two	Root + Shoot	0.5 - 5.0	8.6	175	0.936
	Shoot	0.5 - 5.0	21.0	15.6	0.894
Three	Root + Shoot	50 - 200	379	1870	0.962
	Shoot	50 - 200	417	102	0.975

[1] K_m, Michaelis constant
[2] V_{max}, maximum uptake rate at saturating Ni^{2+} concentration
[3] Coefficients of determination calculated from double reciprocal plots

whether Ni^{2+} uptake was the result of its behavior as an analog, the rate of Ni^{2+} uptake was measured in the absence and presence of Co^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} (Table II). The uptake rate for whole plants maintained on 1.0 μM $^{63}NiCl_2$ was 0.84 μmol of $^{63}Ni^{2+}$·g dry weight $root^{-1}\cdot hr^{-1}$. Addition of 5.0 μM $^{59}NiCl_2$ reduced this rate to 0.47 μmol·g $root^{-1}\cdot hr^{-1}$. This latter rate would be expected for Ni^{2+} in the presence of ions which have an affinity similar to Ni^{2+} for uptake sites. A reduction in uptake does not rule out the possibility of other types of complex or allosteric interactions with the carrier complex or protein which could affect Ni^{2+} uptake. Of the ions investigated, Mn^{2+} and Mg^{2+} did not have an inhibiting effect on Ni^{2+} absorption, while Co^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+} inhibited Ni^{2+} absorption. In the presence of the latter four elements, Ni^{2+} uptake was reduced by 25 to 42% of the rate found for control treatments in the absence of competing ions. Similar reductions in the rate of transport of Ni from roots to shoots were observed (shoots only, Table II) for those ions affecting Ni^{2+} absorption by roots of the intact plants.

To determine which, if any, of the ions shown to inhibit Ni^{2+} uptake were acting as functional analogs, the interactions of Cu^{2+}, Zn^{2+}, Fe^{2+}, and Co^{2+} with Ni^{2+} were analyzed using double reciprocal plots. The kinetic behavior of competition were measured for 21-day-old plants using 1 to 5 μM Ni^{2+} solutions in the absence or presence of 5 μM Cu^{2+}, Zn^{2+}, Fe^{2+}, and Co^{2+}. These competition studies showed Cu^{2+} to be a competitive inhibitor with respect to Ni^{2+} uptake (Fig. 3). Calculated K_m and K_i values were 6.1 and 9.2 μM, respectively. Similar kinetic behavior was observed for Zn^{2+} which was shown to inhibit Ni^{2+} uptake competitively, with K_m and K_i values of 6.7 and 24.4 μM, respectively. Since the K_i values for Cu and Zn represent the inhibitor concentration necessary to double the slope of the 1/v *versus* 1/[S] plot, Cu^{2+} appears to be a better competitor of Ni^{2+} than Zn. The affinity of these carrier sites for Cu^{2+} is about 60% of that for Ni^{2+}, while their affinity for Zn^{2+} is only 25% that of Ni^{2+}. Similar kinetic analysis of the behavior of Fe^{2+} and Co^{2+} in the presence of Ni^{2+} failed to resolve their mode of inhibition, since classical competition kinetic relationships were not observed. Kinetic analysis showed Fe^{2+} and Co^{2+} to inhibit Ni^{2+} uptake at Ni^{2+} concentrations below ~4.0 μM, while increasing Ni^{2+} uptake above ~4.0 μM.

Since a number of studies have demonstrated that Cu^{2+} and Zn^{2+} are competitive inhibitors of each other with respect to absorption (2, 3, 5), it is consistent that both are competitive inhibitors of Ni^{2+}. Although the mode of inhibition of Fe^{2+} and Co^{2+} on Ni^{2+} absorption has not been defined, Fe has been shown to inhibit Cu^{2+} and Zn^{2+} uptake by sugarcane leaf tissues (2) at equimolar concentration (0.1 μM) and to inhibit noncompetitively Zn^{2+} uptake in rice (4). Based on limited Ni data and available kinetic data on nutrilites such as Cu^{2+} and Zn^{2+}, there appears to be a common series of processes involved in the absorption of these divalent cations.

Table II. Effect of nutrient cations on the uptake of nickel by 15-day-old soybean plants

Treatment[2]	Nickel Accumulation Rate[1] Whole Plant (μmol Ni^{2+}/g dry wt root·hr)	Shoot Only (μmol Ni^{2+}/g dry wt root·hr)
1.0 μM Ni^{2+}	0.84 ± 0.09	0.0192 ± 0.0021
1.0 μM Ni^{2+} + 5.0 μM Ni^{2+}	0.47 ± 0.01	0.0087 ± 0.0003
1.0 μM Ni^{2+} + 5.0 μM Cu^{2+}	0.49 ± 0.01	0.0119 ± 0.0001
1.0 μM Ni^{2+} + 5.0 μM Zn^{2+}	0.59 ± 0.01	0.0110 ± 0.0025
1.0 μM Ni^{2+} + 5.0 μM Fe^{2+}	0.56 ± 0.02	0.0049 ± 0.0001
1.0 μM Ni^{2+} + 5.0 μM Co^{2+}	0.63 ± 0.01	0.0111 ± 0.0036
1.0 μM Ni^{2+} + 5.0 μM Mn^{2+}	0.84 ± 0.01	0.0211 ± 0.0008
1.0 μM Ni^{2+} + 5.0 μM Mg^{2+}	1.04 ± 0.06	0.0231 ± 0.0094

[1] Mean ± S.D. for three replicate samples
[2] 1.0 μM Ni^{2+} solution traced with $^{63}Ni^{2+}$ (0.69 μCi/μmol Ni^{2+})

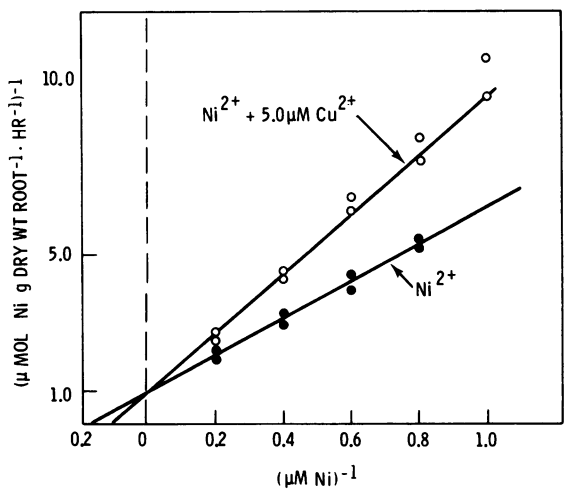

FIG. 3. Double reciprocal plot of uptake data for nickel in the absence and presence of 5.0 μM Cu^{2+} using 21-day-old intact soybean plants.

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