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Does Iron Deficiency in *Pisum sativum* Enhance the Activity of the Root Plasmalemma Iron Transport Protein?

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ABSTRACT

Roots of Fe-sufficient and Fe-deficient pea (*Pisum sativum* L.) were studied to determine the effect of Fe-deficiency on the activity of the root-cell plasmalemma Fe^{2+} transport protein. Rates of Fe(III) reduction and short-term Fe^{2+} influx were sequentially determined in excised primary lateral roots using Fe(III)-ethylenediaminetetraacetic acid (Fe[III]-EDTA). Since the extracellular Fe^{2+} for membrane transport was generated by root Fe(III) reduction, rates of Fe^{2+} influx for each root system were normalized on the basis of Fe(III) reducing activity. Ratios of Fe^{2+} influx to Fe(III) reduction (micromole Fe^{2+} absorbed/micromole Fe[III] reduced) revealed no enhanced Fe^{2+} transport capacity in roots of Fe-deficient peas (from the parental genotype, Sparkle) or the functional Fe-deficiency pea mutant, E107 (derived from Sparkle), relative to roots of Fe-sufficient Sparkle plants. Data from studies using 30 to 100 micromolar Fe(III)-EDTA indicated a linear relationship between Fe^{2+} influx and Fe(III) reduction (Fe^{2+} generation), while Fe^{2+} influx saturated at higher concentrations of Fe(III)-EDTA. Estimations based on current data suggest the Fe^{2+} transport protein may saturate in the range of $10^{-4.8}$ to 10^{-4} molar Fe^{2+}. These results imply that for peas, the physiological rate limitation to Fe acquisition in most well-aerated soils would be the root system's ability to reduce soluble Fe(III)-compounds.

Adaptive responses of root systems to whole-plant Fe deficiency have been studied in a number of species. For dicots (and nongraminaceous monocots), Fe deficiency generally promotes a stimulation of Fe(III) reduction capacity, and increases proton extrusion and the release of phenolics (see refs. 5 and 11 for reviews). These responses are thought to enhance the acquisition of Fe by increasing the solubility of Fe(III)-compounds, as well as increasing the concentration of Fe^{2+} at the exterior surface of the root-cell membrane (5, 11). Ferrous iron has been established as the ionic species of Fe which is transported across the plasmalemma of dicot roots (1).

Iron deficiency has also been shown to promote increased Fe^{2+} absorption in various dicotyledonous plants (1, 3, 8, 12). In all cases, however, Fe was supplied to the roots as Fe(III)-chelate, and since this necessitated a reduction of Fe(III) to Fe^{2+} prior to membrane transport, it is not clear whether the reported increases in Fe^{2+} absorption were the result of enhanced Fe^{2+} transport activity, or merely a consequence of enhanced (Fe-deficiency stimulated) Fe(III) reduction which would generate more Fe^{2+} for absorption into the Fe-deficient roots (3). Although it is reasonable to speculate that Fe^{2+} transport activity might be enhanced as an adaptive response to Fe-deficiency stress, we have been unable to confirm this possibility from the current literature.

In the present study, we have attempted to address the question of whether Fe deficiency enhances Fe^{2+} transport activity in dicots, by measuring both Fe(III) reduction rates and rates of Fe^{2+} influx in roots of Fe-sufficient and Fe-deficient pea (*Pisum sativum* L. cv 'Sparkle') and in the single-gene pea mutant, E107 (*Pisum sativum* L. cv 'Sparkle' E107 [*brz brz*]) (4). This mutant was recently shown to be a functional Fe-deficiency mutant (when grown under Fe-adequate conditions), as it exhibits similar root-system physiological responses to those of Fe-deficient Sparkle (3). We have used Fe(III) reduction measurements as a means to normalize the rates of Fe^{2+} influx observed in plants grown under different Fe regimes. This method has allowed us to make more meaningful Fe^{2+} influx comparisons, and has enabled us to assess the influence of Fe-deficiency stress on the activity of the plasmalemma Fe^{2+} transport protein. Results of this analysis are used to discuss the rate-limiting step(s) in the overall Fe acquisition process.

MATERIALS AND METHODS

Plant Material and Culture

Two genotypes of pea (*Pisum sativum* L.) were used in the present study: a parent line (cv 'Sparkle') obtained from a commercial source, and a mutant line (cv 'Sparkle' E107 [*brz brz*]) which was generated via mutagenesis of cv 'Sparkle' with ethylmethane sulfonic acid (4). Plants of these genotypes were grown in a controlled environment chamber in aerated nutrient solution as previously described (3). Plants were grown to d 15 with either Fe(III)-EDDHA[2] (+Fe-treated plants; Fe was added when nutrient solutions were replaced on d 3, 8, and 10 at 1 μM and d 12 and 14 at 2 μM) or no added Fe (-Fe-treated plants).

Experimental Techniques

Excised primary lateral root units from +Fe- and −Fe-treated plants were used to measure sequentially a rate of

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[2] Abbreviations: EDDHA, *N,N'*-ethylenebis[2-(2-hydroxyphenyl)-glycine]; BPDS, bathophenanthrolinedisulfonic acid.

Fe(III) reduction and a rate of Fe^{2+} influx for each root unit. The root units consisted of a short segment of the primary root (0.5 cm long; excised from the basal end of the root system) which had three to six intact primary lateral roots attached. The chemical solutions and basic methodologies used for measuring Fe(III) reduction and Fe^{2+} influx (including the technique for removing ^{59}Fe[III]-precipitates from the cell wall space) have been previously described (3).

In the present study, the standard protocol for the reduction/influx experiments was to excise the roots, rinse them in aerated nutrient solution for no more than 5 min, and then place them in plexiglass tubes again in aerated nutrient solution. After approximately 5 min, this solution was replaced with the Fe(III) reduction assay solution (which contained Fe[III]-EDTA and Na_2-BPDS; ref. 3), and after 15 min, aliquots were removed for subsequent spectrophotometric analysis to determine the Fe(II)-$BPDS_3$ concentration. Immediately after removing an aliquot of solution, the assay solution was evacuated from the plexiglass tube. Subsequently, each root unit was rinsed with two changes of nutrient solution over a total period of 10 to 12 min; the final rinse was then replaced with nutrient solution containing ^{59}Fe-labeled Fe(III)-EDTA (specific activity: 18.5 kBq $mmol^{-1}$) and 5 mM Mes buffer (pH 5.0). After an uptake period of 20 min, the roots were rinsed, processed (this included the removal of precipitated Fe[III] with a sodium dithionite desorption technique), and counted as previously reported (3). Concentrations of Fe(III)-EDTA for these experiments ranged from 30 to 400 μM; the Na_2-BPDS concentration was 300 μM in all cases. For a given root unit, equivalent Fe(III)-EDTA concentrations were used for the reduction and influx measurements. It should be noted that the exposure of roots to Na_2-BPDS had no deleterious effects on Fe^{2+} influx, as separate experiments revealed similar rates of Fe^{2+} influx with or without the initial Na_2-BPDS treatment for each growth type.

Values of Fe(III) reduction (μmol Fe[III] reduced g fresh $weight^{-1}$ h^{-1}) were either correlated with Fe^{2+} influx determinations (μmol Fe^{2+} g fresh $weight^{-1}$ h^{-1}), or were used to calculate ratio values of μmol Fe^{2+} absorbed/μmol Fe(III) reduced for each root unit. Since these two physiological parameters were not, and could not, be measured simultaneously, it was necessary to establish the rate of Fe(III) reduction which occurred during the Fe^{2+} influx measurement period. Or, in other words, whether any change in Fe(III) reduction occurred during the second exposure to Fe(III)-EDTA. For this purpose, root units from plants grown identically to those used in the reduction/influx studies were subjected to two consecutive Fe(III) reduction measurements. Basically, root units were selected and carried through the protocol for the reduction/influx experiments as described above, except that the Fe^{2+} influx procedures were replaced with a second Fe(III) reduction assay of 20 min duration. At least three root units of each growth type at each Fe(III)-EDTA concentration were assessed in this manner. Average percentage changes in Fe(III)-reductase activity were calculated for the second reduction measurement of the double reduction experiments. These percentage values were then multiplied by the Fe(III) reduction rates obtained in the first phase of the reduction/influx experiments, to determine the rate of Fe(III) reduction which occurred during the Fe^{2+} influx period (the second phase of the reduction/influx experiments). For Fe(III)-EDTA concentrations of 100 μM or less, no change in the rate of Fe(III) reduction was observed for consecutive measurements; hence, no adjustments of the Fe(III) reduction rates at these concentrations were necessary. For Fe(III)-EDTA concentrations of 200 to 400 μM, a small decrease in Fe(III) reduction (4–13%) was observed during the second reduction measurement. Therefore, rates of Fe(III) reduction determined in reduction/influx experiments at these higher Fe(III)-EDTA concentrations were adjusted to account for the small decreases seen during the consecutive reduction measurements. These adjusted Fe(III) reduction rates were used in all data analyses throughout this report.

In one set of experiments with +Fe-treated E107, Fe^{2+} influx was determined for uptake periods of 5, 10, 20, and 30 min, instead of the usual 20 min. For these experiments, Fe^{2+} influx was determined following a 15 min Fe(III) reduction assay as described in the protocol above. Adjusted Fe(III) reduction rates were obtained as described above after performing double reductions on roots of +Fe-treated E107 plants, with the second reduction assay lasting for 5, 10, 20, or 30 min.

RESULTS

Rates of Fe(III) reduction and Fe^{2+} influx were sequentially measured in excised root units of 15-d-old pea plants, in order to compare the relationship between Fe^{2+} influx and Fe(III) reduction among the various growth types. Ratios of μmol Fe^{2+} absorbed/μmol Fe(III) reduced calculated from data obtained at Fe(III)-EDTA concentrations between 30 and 100 μM (see Table I for specific growth types), revealed a narrow range of average ratio values from 0.162 to 0.187 for 15-d-old plants of the various growth types. The average ratio value for +Fe-treated Sparkle was 0.187 ± 0.007 μmol Fe^{2+} absorbed/μmol Fe(III) reduced; none of the other growth types were found to have calculated ratio values higher than that of +Fe-treated Sparkle (Table I).

When the Fe^{2+} influx and Fe(III) reduction data used to calculate ratio values in Table I (all growth types) were correlated, a highly significant ($r = 0.97$, $n = 24$, $P < 0.01$) linear

Table I. *Ratio Values of μmol Fe^{2+} Absorbed/μmol Fe(III) Reduced for Excised Primary Lateral Roots of 15-d-old Pea Plants Measured at Various Fe(III)-EDTA Concentrations*

Reduction and influx measurements were performed on each root unit during sequential time periods. Numbers represent means ± SE of calculated values for at least four replicates.

Genotype	Growth Treatment	Fe(III)-EDTA	Ratio
		μM	$\frac{\mu mol\ Fe^{2+}\ absorbed}{\mu mol\ Fe(III)\ reduced}$
Sparkle	+Fe	100	0.187 ± 0.007
Sparkle	−Fe	100	0.183 ± 0.006
E107	+Fe	30, 50, 75	0.162 ± 0.004[a]
E107	−Fe	100	0.176 ± 0.003

[a] Calculated ratio values arising from data obtained at three Fe(III)-EDTA concentrations were combined to determine this mean.

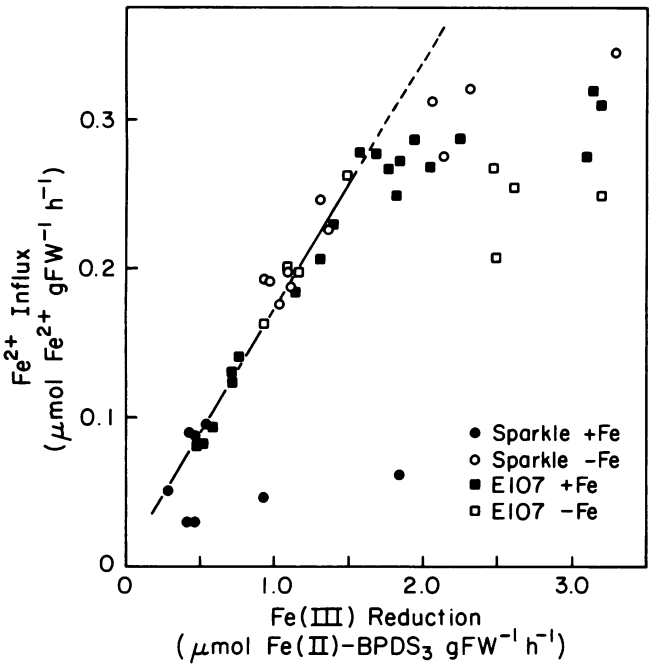

Figure 1. Relationship between Fe^{2+} influx and Fe(III) reduction rates measured in excised primary lateral roots of Fe-sufficient and Fe-deficient growth types of pea. The values for each data point (see inset for growth types) were determined through sequential Fe(III) reduction and Fe^{2+} influx measurements using various concentrations of Fe(III)-EDTA. Reduction and influx values which were used to calculate average ratio values in Table I are plotted in this figure, along with measurements at higher Fe(III)-EDTA concentrations (100 μM for +Fe-treated E107; 200 μM for +Fe-treated E107, −Fe-treated E107, and −Fe-treated Sparkle; 400 μM for +Fe-treated Sparkle). A regression line ($y = 0.168x + 0.005$, $r = 0.98$) is shown for the Table I data set.

relationship was found. The regression line for this set of data ($y = 0.168x + 0.005$, where y is μmol Fe^{2+} absorbed, x is μmol Fe[III] reduced), along with the individual data points for all growth types, are shown in Figure 1. Additionally, data obtained at higher Fe(III)-EDTA concentrations (see Fig. 1 legend) for all growth types are also plotted in Figure 1. All data points from measurements at these higher Fe(III)-EDTA concentrations lie to the right of the regression line in Figure 1, and represent ratio values less than 0.17 μmol Fe^{2+} absorbed/μmol Fe(III) reduced.

The rate of Fe^{2+} influx appears to saturate at increasing Fe(III) reduction rates for all growth types (Fig. 1); Fe^{2+} influx reached a maximum at around 0.28 μmol Fe^{2+} g fresh weight^{-1} h^{-1} for intact primary lateral roots of +Fe-treated E107, −Fe-treated E107, and −Fe-treated Sparkle, and at around 0.05 to 0.09 μmol Fe^{2+} g fresh weight^{-1} h^{-1} for similar roots of +Fe-treated Sparkle. Since it was possible that the high Fe^{2+} influx rates obtained in E107 and Fe-deficient Sparkle, which are associated with increased Fe^{2+} generation, might have led to potentially toxic Fe concentrations in the root cells, we investigated whether Fe^{2+} influx showed signs of inhibition during the 20 min influx period. An Fe^{2+}-influx time course, performed with roots of +Fe-treated E107 at 200 μM Fe(III)-EDTA, revealed that the rate of influx declined slightly during the first 20 min after the initiation of Fe^{2+} uptake, and then drastically from 20 to 30 min (Table II). The ratio, μmol Fe^{2+} absorbed/μmol Fe(III) reduced, showed a somewhat similar trend (Table II). It should be noted that because of this rate decline after 20 min, all Fe^{2+} influx studies were run for 20 min.

In a previous study (3), we demonstrated that when Fe-deficient Sparkle was transferred from −Fe to +Fe growth conditions, an initial increase in the already enhanced Fe(III) reductase capacity was observed 1 d after transfer, which was followed by a gradual return to the low reductase activities measured in Fe-sufficient Sparkle. We thought it would be instructive in the present study to monitor Fe^{2+} influx and Fe(III) reduction throughout this transitional period, as well as during the transitional period when Fe-sufficient plants become Fe-deficient (3). While a transfer from +Fe-treated to −Fe-growth conditions on d 12 promoted an increased rate of Fe^{2+} influx (measured in 100 μM ^{59}Fe-labeled Fe[III]-EDTA) on d 13 through 15 (hatched bars, Fig. 2A), the ratio of Fe^{2+} absorbed/Fe(III) reduced never significantly exceeded the d 12, Fe-sufficient value of 0.15 (Fig. 2C). Similarly, −Fe-treated plants transferred to +Fe-growth conditions showed the highest ratio value on d 15 (open bars, Fig. 2C), when plants of this transitional treatment were exhibiting Fe-adequate responses (note the lowered Fe^{2+} influx and Fe[III] reduction values, Fig. 2, A and B).

DISCUSSION

Iron Transport Response to Iron Deficiency

In dicots and nongraminaceous monocots, Fe-deficiency stress has been shown to promote various root-system physiological responses, which enhance the plant's ability to acquire Fe from the rhizosphere (5, 11). Of these responses, an enhanced capacity to reduce Fe(III)-compounds is of major importance, since Fe is usually present as Fe(III) in well aerated soils, and this Fe must be reduced to Fe^{2+} prior to transport across the root-cell plasmalemma in these types of plants (1). One potential Fe-deficiency response which until now has proven quite difficult to analyze, has been the possibility that the activity of the putative Fe^{2+} transport protein at the root-cell plasmalemma is also enhanced when plants are subjected to Fe-deficiency stress. The problem for re-

Table II. *Time Course Measurements of Fe^{2+} Influx and Fe(III) Reduction for Excised Primary Lateral Roots of 15-d-old +Fe-Treated E107 Pea Plants*

Reduction and influx measurements were performed on each root unit during sequential time periods using 200 μM Fe(III)-EDTA. Numbers represent means ± SE of at least three replicates.

Time interval	Fe^{2+} Influx	Fe(III) Reduction	Ratio
min	*μmol Fe^{2+} / g fresh wt h*	*μmol Fe(III) reduced / g fresh wt h*	*μmol Fe^{2+} absorbed / μmol Fe(III) reduced*
0 to 5	0.327 ± 0.007	3.240 ± 0.103	0.101 ± 0.001
0 to 10	0.307 ± 0.009	2.903 ± 0.057	0.106 ± 0.001
0 to 20	0.300 ± 0.013	3.139 ± 0.030	0.096 ± 0.004
0 to 30	0.210 ± 0.014	2.812 ± 0.181	0.074 ± 0.002

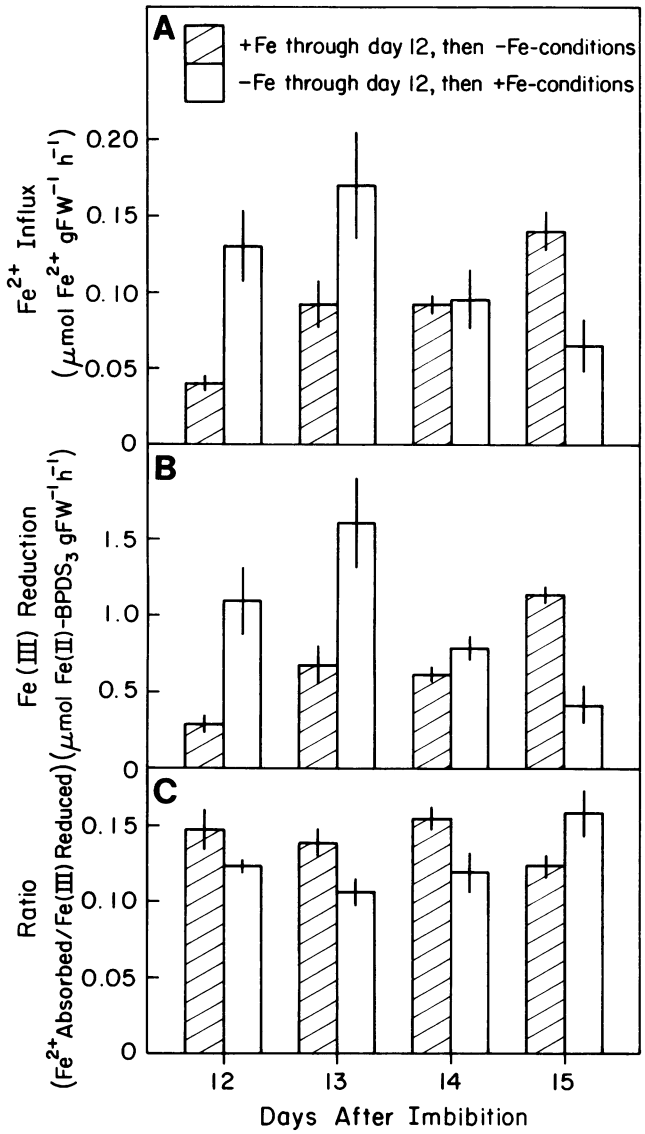

Figure 2. Rates of Fe^{2+} influx, rates of Fe(III) reduction and ratios of Fe^{2+} absorbed/Fe(III) reduced for roots of Sparkle pea plants in transition from Fe-sufficiency to Fe-deficiency, or from Fe-deficiency to Fe-sufficiency. Reduction and influx measurements were conducted sequentially in excised primary lateral roots using 100 μM Fe(III)-EDTA. Hatched bars: d 12, +Fe-treated plants; d 13, 14, and 15, +Fe-treated plants were transferred and maintained on −Fe-growth conditions beginning at the end of d 12. Open bars: d 12, −Fe-treated plants, d 13, 14, and 15, −Fe-treated plants were transferred and maintained on +Fe-growth conditions beginning at the end of d 12. Error bars indicate standard error of the mean for at least four replicates.

searchers has been that supplying the transported ionic species, Fe^{2+}, in a nonlimiting supply in aerated solutions has been quite difficult. Previous investigations of short-term Fe^{2+} influx in dicots have had to utilize an Fe(III)-chelate (instead of free Fe^{2+}) as the Fe source (3), thus forcing researchers to monitor the two-step process of Fe(III) reduction and Fe^{2+} influx, rather than Fe^{2+} influx by itself.

In the present study, both the rate of Fe^{2+} influx as well as the rate of Fe(III) reduction were determined for root units of each growth type. If Fe-deficiency enhances the activity of the root-cell plasmalemma Fe^{2+} transport protein, one would expect to find a higher ratio of Fe^{2+} absorbed/Fe(III) reduced in the Fe-deficient plants. Our calculations of this ratio in 15-d-old plants, however, revealed no major differences between the activity of the Fe^{2+} transport protein in Fe-sufficient and Fe-deficient Sparkle (Table I), or between Fe-sufficient Sparkle and the functional Fe-deficiency mutant, E107 (3) (Table I). Similarly, for Sparkle plants in physiological transition from Fe-sufficiency to Fe-deficiency, or vice versa, ratio values for plants exhibiting Fe-deficiency symptoms (in this case, enhanced rates of Fe[III] reduction) never exceeded the ratio value for Fe-sufficient plants (*i.e.* d 12 +Fe-treated or d 15 −Fe-treated transferred to +Fe-growth conditions, Fig. 2). It appears, therefore, that the Fe^{2+} transport capacity of the root-cell Fe^{2+} transport protein is not enhanced in response to Fe-deficiency in *Pisum sativum.*

It is interesting to note that at low Fe(III)-EDTA concentrations, approximately 17% of the Fe^{2+} ion generated by the Fe(III) reductase was taken up by the root cells (Table I; Fig. 1). While this may seem rather inefficient, this percentage value is probably not a true reflection of the capacity of the Fe^{2+} transport protein. It should be remembered that Fe^{2+} was not supplied to the roots in a constant concentration, as is ideal for influx studies; rather, it was generated at the root surface from Fe(III)-EDTA. Following the release of Fe^{2+} from the reduced Fe(II)-EDTA complex, some of the Fe^{2+} would have diffused to the Fe^{2+} transport protein, while some would have diffused out of the root and through the unstirred layer at the root surface, to the aerated bulk solution where the Fe^{2+} concentration would have been very low. Other processes such as the oxidation of Fe^{2+} through an interaction with EDTA or O_2 (6), or possible precipitation in the cell wall, would also have made some of the Fe^{2+} unavailable for membrane transport. It is also possible that the inclusion of the strong Fe^{2+} chelator, Na_2-BPDS, in the reduction assay might have helped drive the sequence of reactions to the terminal product, Fe(II)-$BPDS_3$, and thus have caused the release of more Fe^{2+} from the reduced Fe(II)-EDTA during the reduction assay, than during the influx assay (WA Norvell, personal communication); this would have lead to an overestimation of the amount of Fe(III)-EDTA reduced during the influx period. Since our understanding of these kinetic and chemical processes is rather limited, it is difficult to make any specific comments about the magnitude of the calculated influx:reduction ratio. It is clear, however, that a significant proportion of the generated Fe^{2+} would not be available to the transport protein in our experimental system. Possibly, only about 17% of the measured Fe(III) reduced was available, since this influx percentage remained constant when Fe^{2+} generation (Fe[III] reduction) was increased, at least at lower

Fe(III)-EDTA concentrations (*e.g.* data for +Fe-treated E107 in Fig. 1).

Is Iron Acquisition Limited by Iron Influx?

Our analysis has shown that Fe^{2+} influx is linearly correlated with Fe(III) reduction, up to a rate of Fe(III) reduction where influx saturates (Fig. 1). This influx saturation appears to be a real response, as it was not due to an Fe-toxicity induced inhibition of continued influx. Experiments with +Fe-treated E107 (using 200 μM Fe[III]-EDTA) showed only a slight decline in the rate of influx during the 20 min influx period (Table II). Furthermore, even during the first 5 min of influx, the calculated ratio value was 0.101 μmol Fe^{2+} absorbed/μmol Fe(III) reduced (Table II), suggesting that rate saturation occurred from the onset of the influx period. It is interesting to note that although Fe^{2+} influx saturated at around 0.28 μmol Fe^{2+} g fresh weight^{-1} h^{-1} in 15-d-old primary lateral roots of +Fe-treated E107, −Fe-treated E107, and −Fe-treated Sparkle, Fe^{2+} influx saturated somewhere between a value of 0.05 and 0.09 μmol Fe^{2+} g fresh weight^{-1} h^{-1} in +Fe-treated Sparkle (Fig. 1). These differences were probably due to the fact that only a fraction of the entire root system was involved in Fe(III) reduction in +Fe-treated Sparkle (see Fig. 3 in ref. 3).

From our present data, along with a number of assumptions, it is possible to estimate the Fe^{2+} concentration at which influx saturates in our experimental system (*i.e.* with the various accompanying ions in the influx solution). To do this, we will use the case of +Fe-treated E107, since most of the E107 root system is known to be involved in Fe(III) reduction (3). For this growth type, Fe^{2+} influx was found to saturate at an Fe(III) reduction rate of around 1.6 μmol Fe(III) reduced g fresh weight^{-1} h^{-1}, when using 100 μM Fe(III)-EDTA (Fig. 1). If we select an average root diameter of 1000 μm, and assume an unstirred boundary layer around the root surface of 500 μm (2), we can state that at steady-state, a net efflux of 1.6 μmol Fe(II)-$BPDS_3$ g fresh weight^{-1} h^{-1} would be measured entering the bulk solution at the outer boundary of the unstirred layer (Fe[III] reduction appears to be linear with time at this concentration; MA Grusak, unpublished results). Inversely, we can state that a net influx of Fe(III)-EDTA of the same magnitude occurs (or a net loss from the bulk solution), since the appearance of Fe(II)-$BPDS_3$ would occur at the expense of Fe(III)-EDTA with a 1:1 stoichiometry. Using these values, and assuming that the flux is cylindrically symmetric around these roots, we can apply the flux equation for radial net ionic flux from the surface of a cylinder (derived in 9) to approximate the steady-state concentration of Fe(III)-EDTA which would be found at the root surface. Using a self-diffusion coefficient of 3.9×10^{-6} cm^2 s^{-1} for Fe(III)-EDTA (10), one can calculate a concentration of 1.3 μM Fe(III)-EDTA at the root surface, given these flux conditions. Since, in our reduction assay, the disappearance of Fe(III)-EDTA at the plasmalemma was not due to influx into the root, but rather a transfer of the Fe moiety to Fe(II)-$BPDS_3$, our calculation would suggest a root surface Fe(II)-$BPDS_3$ concentration of 98.7 μM. Hence, for the situation without BPDS, the instantaneous concentration of Fe^{2+} at the root surface could be as high as 100 μM, and the level at which Fe^{2+} saturates the Fe^{2+} transport protein would be on the order of 10^{-4} M. Alternatively, if only 17% of the reduced Fe(III) is available to the transport protein, as discussed earlier, the protein might saturate in the range of 17 μM ($10^{-4.8}$ M) Fe^{2+}.

In well aerated soils, the measured level of total soluble Fe in soil solution is generally in the range of 10^{-8} to 10^{-6} M (11), with free Fe^{2+} levels generally $<10^{-11}$ M (7). If Fe^{2+} influx saturates in the range of $10^{-4.8}$ to 10^{-4} M Fe^{2+}, and our previous analysis indicates that concentrations of Fe(III)-compounds between 10^{-8} and 10^{-6} M would yield Fe^{2+} concentrations also in the range of 10^{-8} to 10^{-6} M at the root surface, it is clear that Fe(III) reduction would be the limiting physiological step in the overall Fe acquisition process in most aerated soils. This may explain why the activity of the Fe^{2+} transport protein *per se* does not appear to be enhanced in response to Fe-deficiency stress. Apparently, the capacity of the Fe^{2+} transport protein and/or the number of Fe^{2+} transport proteins per unit area of plasmalemma may be adequate to transport any additional Fe^{2+} generated by the plasmalemma Fe(III) reductase, at least in the range of Fe(III) concentrations found in normal soils and especially in Fe-deficient soils.

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