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Mechanism of Iron Uptake by Peanut Plants[1]

I. Fe^{III} REDUCTION, CHELATE SPLITTING, AND RELEASE OF PHENOLICS

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ABSTRACT

Iron deficiency in peanuts (*Arachis hypogeae* L.) caused an increase in release of caffeic acid, a higher rate of Fe^{III} reduction, and increased rates of both Fe^{III} chelate splitting and iron uptake.

Experiments on Fe^{III} reduction by phenolics (*in vitro* experiments) and by roots of Fe-deficient peanuts exclude the direct involvement of released phenolics in Fe^{III} reduction by roots: Fe^{III} reduction by phenolics had a pH optimum higher than 8.0 and was strongly dependent on the concentration and the stability of the supplied Fe^{III} chelates. In contrast, Fe^{III} reduction by roots of Fe-deficient peanuts had a pH optimum of about 5.0 and was less dependent on the stability of the supplied Fe^{III} chelates. Furthermore, the observed release of phenolics into nutrient solution would have to be at least 200 times higher to attain the reduction rates of roots of Fe-deficient peanuts. The results of these experiments support the idea of an enzymic reduction of Fe^{III} on the plasmalemma of cortical cells of roots.

Iron deficiency in the so-called 'Fe-efficient' plant species causes various morphological and physiological changes in the roots. These changes include the formation of rhizodermal transfer cells (7), the accumulation of reducing substances in the rhizodermis, and an enhanced proton efflux: they facilitate iron uptake (2, 14).

Fe^{III} reduction was shown to be an obligatory step in the iron uptake by Fe-efficient species (3). Subsequent studies on the reducing capacities of roots under iron deficiency have considerably increased our knowledge of the regulation of iron uptake in higher plants (9, 11, 15).

Despite this progress, the mechanism of Fe^{III} reduction is unknown: two opposing hypothesis have been suggested. According to Brown and Ambler (2) and Olsen *et al.* (12), iron deficiency causes a release of reducing substances from the roots. These substances reduce Fe^{III} in the apparent free space of the roots and/or in the external solution. This reduction is followed by uptake of Fe^{II} into the root cells. Release of reducing substances under iron deficiency is reported under certain conditions such as acidification of the external solution by enhanced H^+-efflux from roots (10, 11) or by the addition of acetic acid (12). As an alternative to the model of Fe^{III} reduction by released reducing substances, Chaney *et al.* (3) and Bienfait *et al.* (1) put forward a hypothesis of an enzymic iron reduction at the outer surface of the plasmalemma of the cortical cells.

In the present paper, we investigate the relative merits of these two hypotheses. Experiments were carried out where reduction rates of chelated Fe^{III} were determined either *in vivo* with roots of Fe-deficient peanut plants or *in vitro* with various reducing compounds such as phenolics or ascorbic acid. The *in vitro* experiments allowed us to calculate the amounts of the reducing substances which would have to be released by the roots to account for the rates of Fe^{III} reduction observed with roots. Additional experiments examining the uptake of double labeled FeEDDHA[2] ($^{59}Fe[^{14}C]$EDDHA) enabled us to place the step of Fe^{III} reduction into an overall model for the uptake of iron from Fe^{III} chelates by roots.

MATERIALS AND METHODS

Growth of Plants. Seeds of peanuts (*Arachis hypogaea* L. cv A 124B) were germinated in a quartz sand:peat mixture (1:1) with a pH of about 5.5. After 3 d, the seedlings were transferred to nutrient solutions in 15-L plastic tubs under transparent sheeting, and after a further 4 d, to 5-L black plastic pots (four plants/pot).

Nutrient solutions were continuously aerated and had the following composition (M): $Ca(NO_3)_2$, 2.0×10^{-3}; K_2SO_4, 0.75×10^{-3}; $MgSO_4$, 0.65×10^{-3}; KH_2PO_4, 0.5×10^{-3}; H_3BO_3, 1.0×10^{-5}; $MnSO_4$, 1.0×10^{-6}; $CuSO_4$, 5.0×10^{-7}; $ZnSO_4$, 5.0×10^{-7}; $(NH_4)_6Mo_7O_{24}$, 5.0×10^{-8}: the pH was daily adjusted to 6.1 to 6.3 with NaOH. For the control plants, Fe was supplied as 0.1 mM FeEDTA; for the plants grown under iron deficiency, no iron was added to the nutrient solution. After 13 to 14 d growth, the Chl content in shoots of control and Fe-deficient plants was 8.4 to 8.8 and 2.6 to 3.0 mg/g dry weight, respectively.

Plants were grown in a growth chamber with the following conditions: day/night, 16/8 h; light intensity, 70 w/m^2 (fluorescent tubes, Osram-L 40 W/25 and 40 W/77 Fluora); temperature, 25°C; and RH, 65 to 75%.

Measurement of Fe^{III} Reduction. Fe^{III} reduction was determined by the method of Chaney *et al.* (3) using BPDS reagent (0.3 mM). This compound forms a red colored complex with Fe^{II} but not with Fe^{III}. Assays for Fe^{III} reduction contained a source of Fe^{III} ($FeCl_3$, FeEDTA, FeHEDTA, Fe citrate) at concentrations between 5 μM and 5 mM, in a nutrient solution which was devoid of other micronutrients and which was adjusted to pH 6.0 with 10 mM Hepes buffer. Fe desferal, a derivate of the sideramine of *Streptomyces pilosus*, as Fe^{III} chelate was included because of its extremely high chelate stability. After incubation for 15, 30, 60, and 120 min in darkness at 24°C, the absorbance of $Fe(BPDS)_3$ was measured spectrophotometrically at 535 nm. Rates of reduction by roots (0.8–1.2 g fresh weight/plant) of intact peanut plants was measured using 100 ml of assay solution in 200 ml Erlenmeyer flasks on a shaking machine (*in vivo* experiments). Fe^{III} reduction

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[2] Abbreviations: FeEDDHA, ferric ethylenediaminedi (*o*-hydroxyphenylacetate); BPDS, 4,7-di(4-phenylsulphonate)-1,10-phenanthroline; FeHEDTA, ferric *N*-(2-hydroxyethyl)ethylenediaminetriacetate; TPTZ, 2,4,6-tripyridyl-*S*-triazine; C.E.C., cation exchange capacity.

by various reducing substances (0.1 mM) was assayed in glass vials containing 10 ml assay solution (*in vitro* experiments). Solutions of reducing substances were freshly prepared for each experiment.

Release of Reducing Substances by Roots. Rates of release of reducing substances from roots into the nutrient solutions were determined according to Olsen *et al.* (12) colorimetrically with TPTZ, which forms a blue colored complex with Fe^{II}. A 1-ml aliquot of 1.0 M sodium acetate (pH 4.0) was added to 20 ml nutrient solution: then 1.0 ml $FeCl_3$ (1.0 mM) and 3.0 ml TPTZ (1.0 mM) were added. After mixing, the solution was kept in darkness at 24°C for 20 h. The absorbance of $Fe(TPTZ)_2$ was measured spectrophotometrically at 593 nm. The release of reducing substances was calculated as caffeic acid equivalents using a calibration curve with increasing concentrations of caffeic acid. Under the present experimental conditions, 1 μmol caffeic acid reduced 8 μmol Fe^{III} to Fe^{II}. Because of an occasional microbial breakdown of caffeic acid in aerated nutrient solution without roots after 10 to 20 h, experiments to determine the release of reducing substances were restricted to 10 h. During this 10-h period, there was no detectable breakdown of the released reducing substances at pH values from 3.5 to 6.5.

Identification of Released Reductants. To check whether the released reductants contain *o*-diphenols, Arnow reagent (6) was added to samples of the nutrient solution. Particular phenolics were identified using a HPLC apparatus with a 250 × 4.6 mm ZORBAX ODS column (Du Pont de Nemours). The method involved isocratic reverse-phase chromatography, in which the mobile phase was acetic acid:acetonitrile:water (2:35:200), maintained at a flow rate of 1.0 ml/min. Prior to loading the reductants onto the column, samples of nutrient solution were concentrated at 40°C under low pressure and extracted with diethyl ether. The ether phase was evaporated to dryness at room temperature under N_2 gas and the residue was redissolved in 80% methanol (adjusted to pH 3.0 with HCl) for loading the column. Eluted phenolics were detected with an absorbance detector at 254 nm and were identified by comparing their retention times with the retention times of single pure phenolics and mixtures of pure phenolics.

Uptake and Translocation of $^{59}Fe[^{14}C]EDDHA$. $^{59}Fe[^{14}C]EDDHA$ was prepared 2 d before the uptake experiment by mixing $^{59}FeCl_3$ (37 GBq/mol Fe) and [^{14}C]EDDHA (sodium salt, 37 GBq/mol EDDHA) and adjusting the pH to 6.2 by addition of NaOH. Immediately prior to the uptake experiments, the labeled FeEDDHA solution was passed through a membrane filter (0.01 μm) to ensure the removal of colloids of ^{59}Fe hydroxides. For the uptake experiment with double labeled FeEDDHA, the nutrient solution was devoid of other micronutrients. At the end of the uptake period (10 h), the roots were washed with Fe-free nutrient solution for 15 min at 25°C. Plants were then separated into roots and shoots. The ^{14}C activity in the dry material was determined using combustion equipment (Oxidizer, Packard Instruments GmbH) and ^{59}Fe was determined in the recovered ash after dissolving the ash in 3.0 M HCl. The activity of both ^{14}C and ^{59}Fe was measured by the liquid scintillation method. As synthetic chelators are not readily metabolized in plants (5), the ^{14}C content was used to calculate the chelator content and the Fe/chelator ratio in the plants.

C.E.C. and Chl Content. C.E.C. was determined according to Crooke (4) and Chl of freeze-dried shoot samples by spectrophotometry (652 nm) after homogenization with a blender (Ultraturrax) in acetone:water mixture (4:1).

RESULTS

Release of Phenolics and Fe^{III} Reduction by Roots. Lowering the pH of the nutrient solution below 4.4 by addition of H_2SO_4 caused a substantial release of reducing substances. This release was 10 to 15 times higher in chlorotic than in green plants (Fig. 1). The released reducing substances were identified mainly as *o*-diphenolics (>90%) of which caffeic acid was the dominating phenolic (>85%).

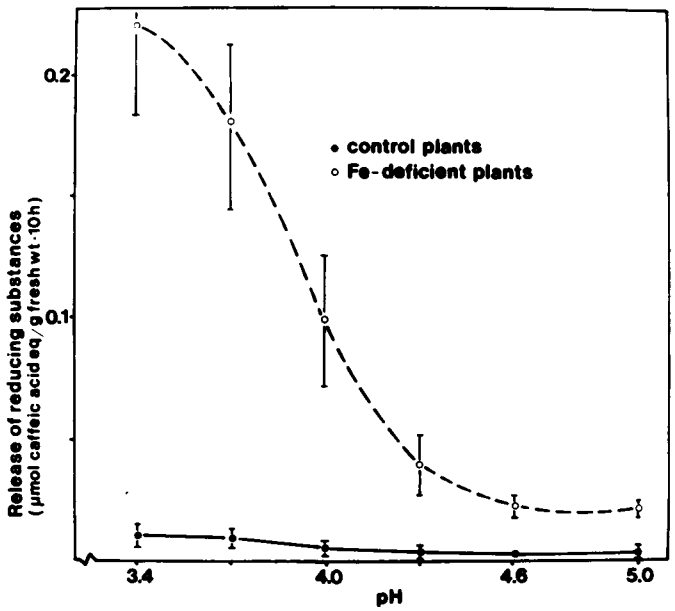

FIG. 1. Release of reducing substances by roots of peanut plants into nutrient solutions of different pH. Reducing substances were determined in the nutrient solution with TPTZ after leaving the peanut plants with the different iron preculture in a newly prepared nutrient solution under aeration for 10 h. Each value represents the mean of four replicates ± SD.

Table I. *Effect of Iron Nutritional Status and the Addition of Phenolic Compounds on Reduction of Chelated Iron by Roots of Peanut Plants*

Assay solution contained 0.3 mM BPDS, 0.1 mM FeEDTA, and 0.1 mM phenolics. Each value is the mean of five determinations ± SD.

Treatment	Fe^{III} Reduction		
	pH 4.0	pH 5.0	pH 6.5
	μmol Fe^{II}/g fresh wt·h		
Fe-adequate plants			
Control	0.12 ± 0.05	0.05 ± 0.02	0.04 ± 0.01
+ *p*-Coumaric acid	0.10 ± 0.06	0.06 ± 0.03	0.05 ± 0.01
+ Caffeic acid	0.11 ± 0.07	0.06 ± 0.03	0.03 ± 0.02
Fe-deficient plants			
Control	3.52 ± 0.53	4.26 ± 0.72	2.57 ± 0.40
+ *p*-Coumaric acid	3.09 ± 0.46	3.29 ± 0.69	1.92 ± 0.51
+ Caffeic acid	0.43 ± 0.20	2.03 ± 0.93	0.86 ± 0.73

In another *in vivo* experiment, the effect of addition of phenolics into nutrient solutions with different pH was tested (Table I). Again, under iron deficiency, the rate of Fe^{III} reduction was 10 to 20 times higher than in Fe-adequate controls. Addition of phenolics did not cause increased rates of Fe^{III} reduction: on the contrary, rates of Fe^{III} reduction decreased with the addition of the phenolics, particularly at low pH values. This result does not support the idea of a direct involvement of released phenolics in the reduction of chelated Fe^{III}.

Comparison of Fe^{III} Reduction in Roots (*In Vivo*) and by Reducing Substances (*In Vitro*). The relationship between the concentrations of free Fe^{3+}, free chelator, and chelated iron are given by the mass action equation 1:

$$\frac{[Fe^{III}\ \text{chelate}]}{[Fe^{3+}] \cdot [\text{chelator}]} = K_s \qquad (1)$$

with the stability constant K_s. It is clear from equation 1 that the concentration of free Fe^{3+} in solution can be manipulated by: (a)

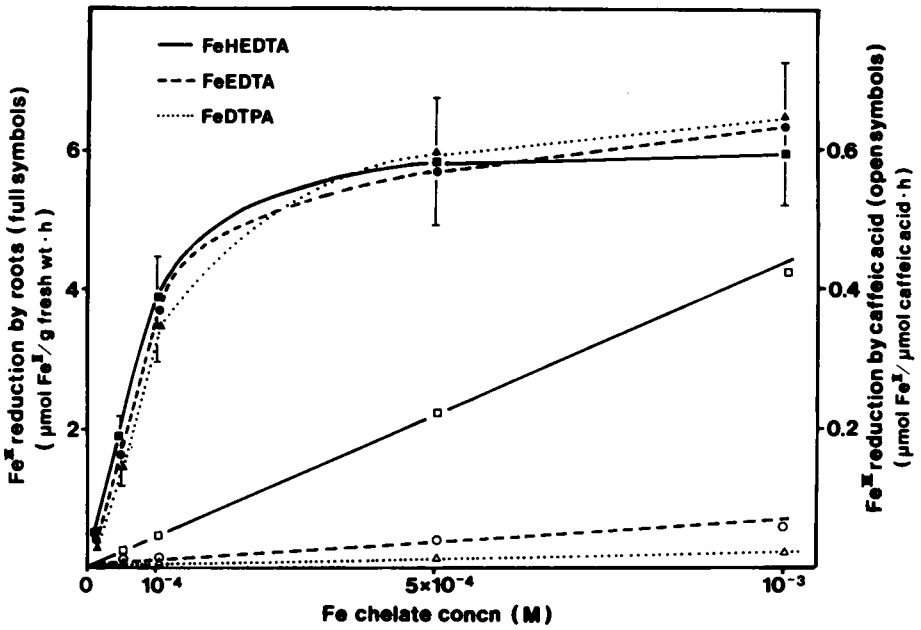

FIG. 2. Effect of increasing concentration of Fe^{III} chelates on rate of Fe^{III} reduction by roots of Fe-deficient peanut plants (●, ■, ▲) or caffeic acid (○, □, △). Fe^{III} reduction was determined with 0.3 mM BPDS and 0.1 mM caffeic acid at pH 6.0. Values represent the mean of five replicates ± SD.

Table II. *Rate of Fe^{III} Reduction by Reducing Substances as Affected by the Stability Constant of Fe^{III} Chelates*

Fe^{III} reduction was determined with 0.3 mM BPDS in nutrient solution at pH 6.0. Concentration of the reducing substances and iron chelates was 0.1 mM.

Fe^{III} Chelate	Stability Constant (K_s)	Fe^{III} Reduction		
		Ascorbic acid	Hydroxylamine hydrochloride	Caffeic acid
		μmol Fe^{II}/mmol reductor·h		
Fe citrate	$10^{11.4}$	364	227	357
FeHEDTA	$10^{19.8}$	1,091	614	46
FeEDTA	$10^{24.2}$	594	29	12
FeDTPA	$10^{28.6}$	48	6	1
Fe desferal	$10^{30.7}$	<1	<1	<1
$FeCl_3$		565	366	542

Table III. *Rate of Fe^{III} Reduction by Roots of Chlorotic Peanut Plants as Affected by the Stability of Fe^{III} Chelates and by Addition of Excess of Free Chelators*

Fe^{III} reduction was determined by addition of 0.3 mM BPDS and the various Fe chelates (0.1 mM) to the roots in aerated nutrient solutions at pH 6.0. Each value is the mean of five determinations ± SD.

Fe^{III} Chelate	Fe^{III} Reduction	
	Without chelator excess	Chelator excess (0.5 mM)
	μmol Fe^{II}/g fresh wt·h	
Fe citrate	0.59 ± 0.12	0.64 ± 0.14
FeHEDTA	3.94 ± 0.55	3.74 ± 0.59
FeEDTA	3.71 ± 0.56	3.80 ± 0.98
FeDTPA	3.70 ± 0.59	3.33 ± 0.77
Fe desferal	<0.05	<0.05
$FeCl_3$	0.49 ± 0.18	

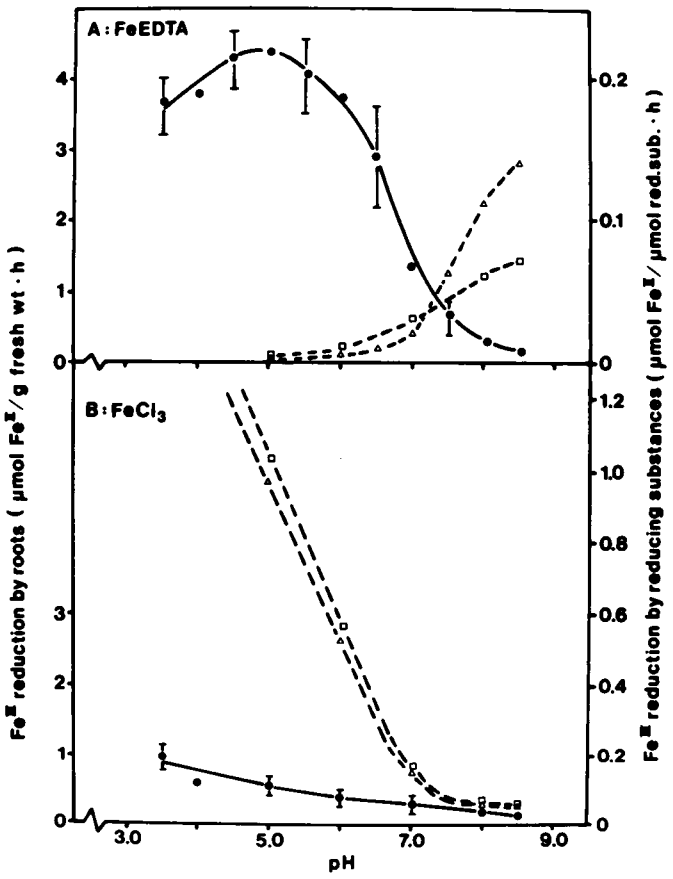

FIG. 3. Effect of pH on rate of Fe^{III} reduction by roots of Fe-deficient peanut plants (●) and reducing substances (△, □). Fe^{III} reduction was determined with 0.3 mM BPDS, 0.1 mM caffeic acid (□), and 0.1 mM chlorogenic acid (△).

Table IV. *Ability of Various Reducing Substances for Fe^{III} Reduction from FeEDTA In Vitro and Theoretical Content of These Reductants in Roots, To Attain Comparable Reducing Rates as Roots of Fe-Deficient Peanut Plants*

Fe^{III} reduction by the various reducing substances was determined with 0.3 mM BPDS and 0.1 mM Fe^{III} EDTA at pH 6.0. For calculation of the theoretical content of reductants in roots, a reduction rate of 3.71 μmol Fe^{II}/g fresh weight·h for Fe-deficient peanut plants was taken.

Reductant	Fe^{III} reduction	Theoretical Content of Reductants in Roots	
	μmol Fe^{II}/μmol reductor·h	*μmol/g fresh wt*	*mg/g dry wt*
Ascorbic acid	0.594	6.3	15
Hydroxylamine hydrochloride	0.029	128.0	128
Hydrochinone	0.016	231.9	332
Gallic acid	0.015	247.3	666
Caffeic acid	0.012	309.2	798
Chlorogenic acid	0.010	371.0	1,883[a]
Ferulic acid	<0.002	>1,000	>3,004[a]
GSH	<0.002	>1,000	>5,626[a]
NADH	<0.002	>1,000	>7,076[a]
NADPH	<0.002	>1,000	>16,075[a]

[a] Theoretical content more than 100% of root weight.

Table V. *Effect of Ground Dry Material (DM) of Roots from Fe-Deficient Peanut Plants on the Rate of Fe^{III} Reduction from FeEDTA by Various Reducing Substances in a Nutrient Solution*

Fe^{III} reduction was measured with 0.3 mM BPDS. Ground root material (DM) was added in amounts comparable to *in vivo* experiments with intact roots (10 mg dry material/10 ml nutrient solution), 0.1 mM FeEDTA, pH 6.0.

Reducing Substance	Fe^{III} Reduction	
	− DM	+ DM
	μmol Fe^{II}/mmol reductor·h	
Ascorbic acid	593	512
Hydroxylamine hydrochloride	24	20
Caffeic acid	8	7

Table VI. *Relationship between the Cation Exchange Capacity of Ground Root Dry Matter from Various Plant Species and the Capacity of this Dry Matter to Adsorb ^{59}Fe and [^{14}C]EDDHA from ^{59}Fe[^{14}C]EDDHA*

Supply of 20 μM ^{59}Fe[^{14}C]EDDHA in a nutrient solution at pH 6.0 under continuous shaking for 10 h. Each value is the mean of four determinations ± SD.

Plant Species	Cation Exchange Capacity	^{59}Fe	[^{14}C]EDDHA
	μeq/g dry wt	*nmol/g dry wt*	
Peanut			
Control (green)	401 ± 39	754 ± 45	ND[a]
− Fe (chlorotic)	384 ± 40	738 ± 47	ND
Sunflower	438 ± 37	683 ± 38	26 ± 10
Corn	138 ± 20	139 ± 13	15 ± 8
Barley	104 ± 13	74 ± 5	ND
Sorghum	89 ± 10	39 ± 3	17 ± 8

[a] Not determined.

changing the total concentration of Fe^{III} chelate; (b) changing the concentration of free chelator; and (c) using chelates of different stability constant.

Two *in vitro* experiments suggest that there is a direct correlation between the rates of Fe^{III} reduction by phenolics and the concentrations of free Fe^{3+} in the solutions. First, the rate of Fe^{III} reduction *in vitro* increased linearly as the concentration of Fe^{III} chelates increased from 10^{-5} to 10^{-3} M (Fig. 2). In addition, reduction of chelated Fe^{III} by various reducing substances as ascorbic acid or caffeic acid was, in general, higher with iron chelates of low stability than with iron chelates of high stability (Table II; Fig. 2).

In contrast, with roots of Fe-deficient peanuts (*in vivo* experiments) there was no direct relationship between concentrations of free Fe^{3+} and rates of Fe^{III} reduction (Table III; Fig. 2). Fe^{III} reduction by roots of peanuts was affected neither by the stability of iron chelates nor by the presence of excess free chelator (Table III). Roots of Fe-deficient peanuts reduced the weak chelate Fe citrate and the strong chelate Fe desferal at relatively low levels and other chelates of varying stabilities (FeHEDTA, FeEDTA, FeDTPA) at uniformly high rates (Table III). Furthermore, there was no linear relationship between rates of Fe^{III} reduction and the concentration of iron chelates (Fig. 2). On the contrary, Fe^{III} reduction by roots showed typical saturation kinetics: rates of Fe^{III} reduction in the low concentration range increased sharply with increasing concentrations of iron chelate but leveled off at higher chelate concentrations.

The results of Figure 2 and Tables II and III suggest different mechanisms for the reduction of chelated Fe^{III} by reducing substances (*in vitro*) and by roots (*in vivo*). This suggestion is further supported by the pH dependence of Fe^{III} reduction (Fig. 3). Reduction of Fe^{III}EDTA by roots of chlorotic peanut plants had an optimum around 5.0, whereas the various reducing substances had pH optima higher than 7.0 depending on the iron chelate used (Fig. 3A shows values for FeEDTA). On the other hand, reduction of Fe^{III} chloride by reducing substances and roots was facilitated by lowering the pH (Fig. 3B).

The rather poor ability of phenolic compounds and ascorbic acid to reduce Fe^{III}EDTA is illustrated in Table IV. With the exception of ascorbic acid, all other reducing substances (including phenolics such as caffeic acid) reduced Fe^{III} at very low rates compared to the rates by roots of Fe-deficient plants. If it was assumed that these reducing substances were entirely responsible for the reduction by roots, then the theoretical content of reductant required in the roots and/or in the free space of the cortex cells may be calculated (Table IV). With the exception of ascorbic acid, unrealistically high concentrations of reducing substances would be required to sustain the high rates of Fe^{III} reduction by roots of Fe-deficient peanuts. As the content of ascorbic acid in roots of Fe-deficient plants is below 0.3 mg/g dry weight (Bienfait, personal communication) also an involvement of ascorbic acid in Fe^{III} reduction by roots can be excluded. Because iron reduction in roots of Fe-deficient plants takes place primarily in distinct root zones (apical meristems, rhizodermal cells), the content of reducing substances in these root zones would have to be even higher than the calculated average values in Table IV.

Role of C.E.C. of Roots for Reduction and Adsorption of Fe^{III}. The effect of ground dry material from peanut roots on the rate of iron reduction by various reducing substances was studied in order to test whether the negative charges (C.E.C.) of the cell walls are a contributing factor for the differences in rate of Fe^{III} reduction from iron chelates *in vitro* and *in vivo* (Table V). Root dry matter had no enhancing effect on the reduction by these reducing substances. Furthermore, the C.E.C. of peanut roots was not affected by the iron nutritional status and, correspondingly, the ability for ^{59}Fe adsorption from ^{59}FeEDTA was the same in root dry matter of green and chlorotic peanut plants (Table VI). Nevertheless, a comparison of different plant species suggest that the C.E.C. could play a role in Fe adsorption from chelated iron (Table VI). In monocots with low C.E.C., less iron could be

Table VII. *Content of ^{59}Fe and Chelator (^{14}C) in Peanut Plants as Affected by Iron Nutritional Status (Preculture ± 0.1 mM FeEDTA)*

$^{59}Fe[^{14}C]EDDHA$ (20 μM) was supplied to roots of intact peanut plants (14 d old) in nutrient solution (pH 6.2) for 10 h. Values are means of five replicates; SD, 5 to 20%.

Preculture	Chl (Upper Shoot)	Roots			Shoot		
		^{59}Fe	^{14}C	Fe/C	^{59}Fe	^{14}C	Fe/C
	mg/g dry wt	*nmol/g dry wt*		*ratio*	*nmol/g dry wt*		*ratio*
+ Fe	8.57	218	63	3.5	4.1	4.0	1.02
− Fe	2.80	10,423	52	200.4	1,814	4.9	370

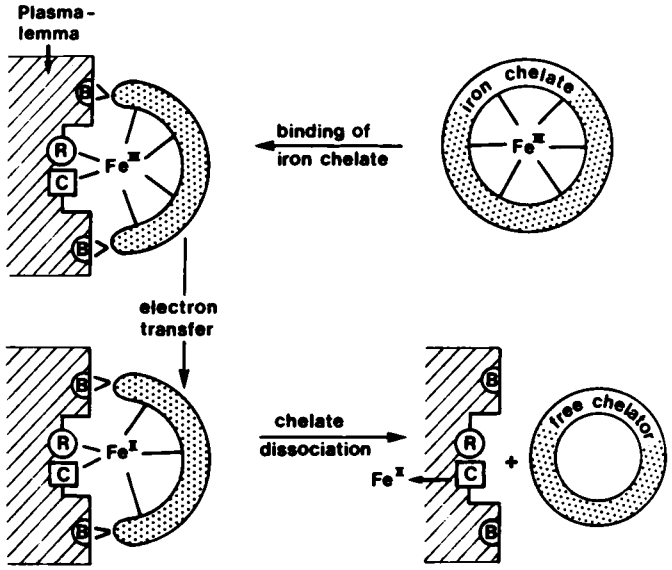

FIG. 4. Sorption of Fe^{III} chelate and Fe^{III} reduction on the plasmalemma. A model for Fe chelate splitting. (B), Specific binding sites; (R), reducing enzyme; (C), carrier for Fe^{II} transport across plasmalemma.

adsorbed onto the cell walls. Dicots however had higher C.E.C. and were more effective in their capacity to bind ^{59}Fe. This increased ^{59}Fe adsorption with increasing C.E.C. is mainly due to chelate splitting as shown with supply of double labeled $^{59}Fe[^{14}C]$ EDDHA to root material of sunflower, corn, and sorghum (Table VI). Therefore, particularly at high C.E.C. (*e.g.* sunflower), the adsorbed ^{59}Fe was predominantly at the unchelated form, which indicates this chelate splitting.

Relationship between Iron Nutritional Status, Chelate Splitting, and Iron Uptake by Roots. Under iron deficiency, roots of peanut plants increased their capacity for the uptake and transport of iron (Table VII). Iron deficiency caused a dramatic increase in $^{59}Fe/[^{14}C]$chelator ratio in both roots and shoots. This increased iron uptake was solely due to an increase in uptake of unchelated ^{59}Fe; uptake of the chelator $[^{14}C]EDDHA$ was hardly affected (Table VII). Clearly, the increased iron uptake coincided with increased ability to split the iron chelate.

DISCUSSION

Under iron deficiency, peanut plants show physiological adaptations in roots comparable to those of sunflower (14), that is, increased rates of Fe^{III} reduction (Table I) and iron uptake (Table VII). Simultaneously, proton efflux is enhanced and the formation of rhizodermal transfer cells can be observed (Marschner and Römheld, unpublished). Thus, peanut can be classified as a so-called Fe-efficient plant species (16).

For the high uptake rate of iron in Fe-efficient plant species under conditions of iron deficiency, reduction of Fe^{III} is required as an obligatory step (3, and unpublished data). Brown and Ambler (2) and Olsen *et al.* (12) proposed a direct involvement of reducing substances such as phenolics (*e.g.* caffeic acid) for this Fe^{III} reduction. In sunflower under conditions of iron deficiency, there was an accumulation of *o*-diphenolics (14), as well as a release of reducing substances into the nutrient solution (10). A similar release was reported for soybeans (2, 3) and tomatoes (12). In tomato, caffeic acid and three other *p*-coumaric acid derivates were released into the nutrient solution (12). In agreement with these results, we show here that roots of Fe-deficient peanut plants release more reducing substances into the nutrient solution than Fe-adequate control plants (Fig. 1). These reducing substances were identified by HPLC as being mainly caffeic acid (>85%). However, a substantial increase in release of reducing substances (*e.g.* caffeic acid) under iron deficiency occurred only at pH values lower than 4.5 (Fig. 1). Similar results were also found for tomato (12) and sunflower (9). On the other hand, acidification of the medium was not necessary for *in vivo* reduction of chelated Fe^{III} by roots of Fe-deficient peanuts. Under iron deficiency, peanut plants reduced chelated iron relatively rapidly at pH 7.0, with a maximum at pH 5.0 (Fig. 3). It is therefore unlikely that released phenolics are responsible for enhanced reduction of chelated Fe^{III}.

This conclusion is also supported by other data. Phenolics are much less effective in reducing iron from FeEDTA than roots of Fe-deficient plants. The theoretical amounts of phenolics which would have to be released from the roots into apparent free space to explain the reduction of Fe chelates at pH 6.0 were much too high to suggest any direct involvement of phenolics in Fe^{III} reduction by roots (Table IV). Even at higher pH values (pH 7.0–8.0) where phenolics were more effective in the reduction of chelated Fe^{III} (Fig. 3), unrealistically high amounts of these substances would still have to be released.

The basic differences between the reduction of chelated Fe^{III} by roots of Fe-deficient plants or by reducing substances like phenolics were reflected in the different responses to the pH and the chelate stability (Table II and III) as well as to the concentration of the Fe chelates (Fig. 2). Rates of reduction of chelated Fe^{III} by phenolics were correlated with the concentration of free Fe^{3+} in the solutions (Table II; Fig. 2). We therefore conclude that reducing substances reduced chelated Fe^{III} only after chelate dissociation. The relatively rapid reduction of unchelated iron by reducing substances (Table II) supports this conclusion. Furthermore, we assume that the increased reduction of chelated iron by reducing substances at high pH values (Fig. 3) is the result of the lower chelate stability at these pH values, with a corresponding increased formation of Fe^{3+}. In contrast to the *in vitro* situation, rates of Fe^{III} reduction by roots were not correlated with the concentration of Fe^{3+} (Table III; Fig. 2).

The extremely high reduction rates of Fe-deficient roots compared to phenolics suggest that dissociation of chelated iron takes place prior to Fe^{III} reduction. Stewart and Leonard (19) have proposed that the negative charges in the cell walls (C.E.C.) may favor chelate dissociation (chelate splitting). In the present paper, it has been shown that two Fe-efficient species (peanut and sunflower) have much higher abilities to bind previously chelated iron to their cell walls than Fe-inefficient species (Table VI).

However, in peanuts, this enhanced chelate splitting was still not sufficient to promote the reduction of Fe^{III} by phenolics (Table V).

Even if it was assumed that all Fe^{III} of a supplied Fe chelate was easily accessible as inorganic Fe^{III} in the apparent free space for reduction by phenolics, the measured release of phenolics would still be too low to explain the observed rates of Fe^{III} reduction of roots. For example, at pH 5 the release of phenolics (caffeic acid) by Fe-deficient peanut plants would have to have been 200 times higher than measured (Fig. 1) to obtain the reduction rate of roots at this pH (Table I). Clearly, the results are inconsistent with the idea of a direct involvement of phenolics in the measured reduction of chelated Fe^{III} by roots of of Fe-deficient peanut plants. On the contrary, the data suggest that Fe^{III} is reduced at highly active sites, perhaps by an enzyme as proposed by Chaney *et al.* (3) and Bienfait *et al.* (1). The saturation kinetics of reduction of chelated Fe^{III} by roots (Fig. 2) suggest a limited number of these reaction sites for the electron transfer. These reaction sites, however, must presumably have a high efficiency in order to explain the extremely high reduction rates of Fe-deficient roots compared to phenolics. We suggest here that the reduction of Fe^{III} chelate by roots is probably preceded by a chelate binding and weakening of chelate bonds (partial splitting of chelate bonds), which in turn leads to a facilitated electron transfer in the subsequent reduction process. Binding of chelated Fe^{III} prior to reduction would explain the lack of correlation between chelate stability and Fe^{III} reduction by roots (Table III). In the case of ferric citrate, the formation of polymer particles (18) may explain this lack of correlation.

The rapid increase in reduction and uptake of iron from iron chelates by roots of Fe-deficient peanuts was not associated with a corresponding increase in chelator uptake (Table VII). Therefore, binding and reduction of the iron chelates would have to occur at the outer surface of the plasmalemma of root cells. The primary features of this modified model are illustrated in Figure 4. Under iron deficiency, either the affinity of the reductase (R) or the number of binding (B) and reducing sites increases. The formation of rhizodermal transfer cells with corresponding increase in plasmalemma surface (7) would support the latter assumption.

Iron reduction at the actual uptake sites (plasmalemma) of the proposed mechanism (Fig. 4) would have various advantages compared to iron reduction by phenolics released from the roots. Most important, this mechanism would bypass wasteful reoxidation and/or decomposition of the phenolics by microorganisms and would provide a sensitive and rapid means of regulating iron reduction according to the actual iron nutritional status of the plants. However, one consequence of this proposed mechanism is that a sufficiently high concentration of soluble iron (Fe chelate) would be required in the rooting medium and the apparent free space, respectively. According to Figure 2, a concentration of iron chelate in the range of 10^{-4} to 10^{-3} M would be maximal for iron reduction by roots. In long term studies, however, much lower concentrations of iron chelates (<10 μM) are sufficient to cover the iron demand for optimal growth (8, and unpublished data). In soil solutions, depending upon the pH and the redox potential of the soils, a high proportion of iron can be observed as complexed iron (17). Concentrations of complexed iron in soil solutions as hydroxamate siderophores between 10^{-8} and 10^{-7} M were found by Powell *et al.* (13). It is very likely that, within the rhizosphere, the concentration of soluble iron might be higher as a result of low molecular organic compounds (chelators) produced by microorganisms and/or roots. In this context, phenolics released by roots might have particular importance as chelators for Fe^{III}. The role of released phenolics (*e.g.* caffeic acid) for iron mobilization in soils will be discussed in a following paper (unpublished data).

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