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Mechanism of Short Term Fe^{III} Reduction by Roots[1]

EVIDENCE AGAINST THE ROLE OF SECRETED REDUCTANTS

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ABSTRACT

The hypothesized role of secreted reducing compounds in Fe^{III} reduction has been examined with Fe-deficient peanuts (*Arachis hypogaea* L. cv A124B). Experiments involved the exposure of roots to (a) different gas mixtures, (b) carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and (c) agents which impair membrane integrity.

Removing roots from solution and exposing them to air or N_2 for 10 minutes did not result in any accumulation in the free space of compounds capable of increasing rates of Fe^{III} reduction when roots were returned to solutions. On the contrary, exposing roots to N_2 decreased rates of Fe^{III} reduction. CCCP also decreased rates of Fe^{III} reduction.

Acetic acid and ethylenediaminetetraacetic acid (disodium salt) (EDTA) impaired the integrity and function of the plasma membranes of roots of Fe-deficient peanuts. That is, in the presence of acetic acid or EDTA, there was an efflux of K^+ from the roots; K^+ (^{86}Rb) uptake was also impaired. Acetic acid increased the efflux from the roots of compounds capable of reducing Fe^{III}. However, both acetic acid and EDTA caused rapid decreases in rates of Fe^{III} reduction by the roots. In addition to peanuts, acetic acid also decreased rates of Fe^{III} reduction by roots of Fe-deficient sunflowers (*Helianthus annuus* L. cv Sobrid) but not maize (*Zea mays* L. cv Garbo).

These results suggest that, at least in the short term, the enhanced Fe^{III} reduction by roots of Fe-deficient plants is not due to the secretion of reducing compounds.

Fe^{III} reduction by roots may be rapidly assayed in nutrient solutions using the chelator BPDS[4], which has high affinity for Fe^{II} and forms the red colored $[Fe^{II}(BPDS)_3]^{-4}$ complex (5). In 'Fe-efficient' species like sunflowers, peanuts, and soybeans, Fe deficiency causes increased rates of Fe^{III} reduction by roots and the acidification of the external medium (5, 18, 22). The adaptive significance of increased Fe^{III} reduction is clear because in Fe-efficient species, reduction of Fe^{III} to Fe^{II} is an essential prerequisite to Fe uptake (5). In contrast in 'Fe-inefficient' species like maize, Fe deficiency does not cause increased rates of Fe^{III} reduction (21).

Two models have been proposed for the mechanism of Fe^{III} reduction by roots. Olsen and colleagues (19, 20) suggest that Fe^{III} reduction occurs in the rhizosphere, and that phenolic compounds secreted from the roots are the source of electrons. In support of this, it was found that when plants were grown in nutrient solutions which had pH values below 4.0, compounds accumulated in the nutrient solutions which were capable of reducing inorganic Fe^{III} to Fe^{II} over a 20- to 24-h period. The release of reductants in the nutrient solutions was greater with Fe-deficient than Fe-adequate plants (2, 17, 20, 23). An investigation with HPLC suggested that the major secreted reductants were caffeic acid and *p*-coumaric acid (20).

Other investigations highlight a major inadequacy of the 'Olsen' model: phenolics reduce Fe^{III} much too slowly to explain the high rates of Fe^{III} reduction by roots of Fe-deficient plants (5, 23). Expanding on an earlier suggestion of Chaney *et al.* (5), Bienfait *et al.* (1) have suggested that Fe^{III} might be reduced at the root epidermis by a plasma membrane-bound enzyme ('Fe^{III} reductase'). In this alternative model, electrons are presumably donated to the enzyme by a reducing compound inside the cell; the electrons on the enzyme are then transferred to Fe^{III} at the outer surface of the plasma membrane.

In the present paper, we have attempted to discriminate between these models using two approaches. (a) Plant roots were removed from solution and exposed to air or N_2 to see if reductants accumulated in the free space in sufficient quantities to explain rates of Fe^{III} reduction. (b) Plasma membrane integrity was manipulated using acetic acid or the chelating agent EDTA, both of which cause leakage of organic and inorganic substances from the roots (13, 15, 16). If the Olsen model for Fe^{III} reduction is correct, then factors which increase membrane permeability might increase the efflux of reductants from the root, thereby increasing rates of Fe^{III} reduction. On the other hand if the 'Bienfait/Chaney' model is correct, then increased efflux of reductants from root cells might decrease rates of transfer of electrons to the plasma membrane bound Fe^{III} reductase, thereby lowering rates of Fe^{III} reduction.

MATERIALS AND METHODS

Growth of Plants. Seeds of sunflower (*Helianthus annuus* L. cv Sobrid), peanut (*Arachis hypogaea* L. cv A124B), and maize (*Zea mays* L. cv Garbo) were germinated in moistened sand, peat and sand mixture, or between filter paper as previously described (21–23).

Peanut and sunflower seedlings were transferred on day 3 to aerated nutrient solutions containing (μM): $Ca(NO_3)_2$, 2,000; K_2SO_4, 750; KH_2PO_4, 500; $MgSO_4$, 650; H_3BO_3, 10; $MnSO_4$, 0.1; $ZnSO_4$, 0.05; $CuSO_4$, 0.05; $(NH_4)_6Mo_7O_{24}$, 0.005. The pH of the nutrient solutions was adjusted daily with NaOH to 6.0. 'Low Fe' plants were grown without Fe in the nutrient solution: Fe^{III}EDTA (100 μM) was added to 'high Fe' plants on day 4. Solutions were renewed on day 6. Peanuts were grown with a

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[4] Abbreviations: BPDS, bathophenanthrolinedisulfonate; TPTZ, 2,4,6-tri(2-pyridyl)-s-triazine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

day/night regime of 16/8 h, 27/20°C, with an illuminance of 20,000 lux (incandescent lights, Osram Powerstar HQI-T 2000W-D). Sunflowers were grown under conditions similar to peanuts (22) with an illuminance of 6,000 lux (fluorescent tubes, Osram-L 40W/25 and 40W/77 Fluora). Peanuts were assayed for Fe^{III} reduction on day 8 or day 9; sunflowers were assayed on day 10.

Maize seedlings were transferred on day 4 to aerated nutrient solutions containing (μM): $Ca(NO_3)_2$, 2,000; K_2SO_4, 750; $MgSO_4$, 650; KH_2PO_4, 100; Fe^{III}EDTA, 100; H_3BO_3, 10; $MnSO_4$, 1.0; $ZnSO_4$, 0.5; $CuSO_4$, 0.5; $(NH_4)_6Mo_7O_{24}$, 0.05. Solutions were changed every second day. High Fe maize had Fe^{III}EDTA in their nutrient solutions throughout the growth period; with low Fe maize, Fe^{III}EDTA was omitted from fresh nutrient solutions on day 8. Environmental conditions were as described for peanuts. Senescing seeds of developing maize plants were frequently covered by a thick gelatinous microbial contamination which may have caused Fe^{III} reduction. The seed and seminal roots were therefore excised on day 10. By this time, each plant had four well developed nodal roots. Nodal roots of maize were assayed for Fe^{III} reduction on day 15.

Fe^{III} Reduction by Roots. Reduction of Fe^{III} by roots was determined using BPDS (5). Unless otherwise indicated, assay solutions were buffered with 10 mM Mes or 10 mM acetic acid and brought to pH 5.0 with NaOH. In addition, assay solutions contained 100 μM Fe^{III}EDTA, 300 μM BPDS, and 50 μM 'free' Ca^{2+} (supplied as $CaSO_4$): 182 or 917 μM $CaSO_4$ was required to give 50 μM free Ca^{2+} in solutions buffered with Mes or acetic acid, respectively. The reason for this is that both Mes^- and $acetate^-$ ions bind Ca^{2+}. The required concentrations of $CaSO_4$ were calculated assuming binding constants (log K_1) of 0.7 for $(Ca^{2+}Mes^-)^+$ (12) and 0.5 for $(Ca^{2+}acetate^-)^+$ (7). Concentrations of Mes^- and $acetate^-$ were calculated using the Henderson-Hasselbalch equation.

Assays for Fe^{III} reduction were conducted in the dark at 20 to 22°C with intact plants: roots were carefully inserted into Erlenmeyer flasks and 'rinsed' in 50 μM $CaSO_4$. Flasks were either agitated on a mechanical shaker or were bubbled with air. After 20 to 30 min, the 'rinsing' solutions were replaced by assay solutions of 60 or 150 ml. Seven-ml samples were removed at intervals and filtered. Optical densities were measured in a 1-cm cuvette at 535 nm. Concentrations of Fe^{II} were calculated assuming a molar absorptivity for $[Fe^{II}(BPDS)_3]^{-4}$ of 22,000 (5). In the experiment of Figure 1, rinsed roots were exposed to a flow of air or N_2 for 10 min. Rates of Fe^{III} reduction were then assayed as above.

Markers of Membrane Integrity. Changes in the integrity of the root plasma membranes were indicated by either (a) the net leakage of K^+ from the roots into K^+-free solutions (measured over a 30-min interval), or (b) changes in the uptake of K^+ labeled with ^{86}Rb. In the former case, K^+ concentrations in the assay solutions were measured by flame photometry. In the latter case, roots were incubated for 30 min in solutions containing 0.5 mM K^+ labeled with ^{86}Rb, 50 μM free Ca^{2+} (added as $CaSO_4$), and either 10 mM Mes or 10 mM acetic acid: the pH of the solutions was adjusted to 5.0 with NaOH and the concentration of Na^+ was adjusted to 7.0 mM with NaCl. In some cases, plants had a pretreatment for 30 min in solutions without label. After the incubation treatments, roots were rinsed for 20 min at 2°C with a nonradioactive solution containing 0.5 mM KCl and 50 μM $CaSO_4$. Roots were excised, oven dried, weighed, and ashed at 450°C. The ash was resuspended in 5.0 ml of 1% (w/v) HCl. Radioactivity was determined by liquid scintillation counting.

Efflux of Reducing Compounds. The efflux of reducing compounds from roots of peanuts was determined by measuring the capacity of external solutions to reduce inorganic Fe^{III} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (2). Peanuts were initially grown as previously described. On day 6, plants were tied into bunches of four and grown for a further 2 d in nutrient solutions buffered at pH 5.0 with 10 mM Mes/Na^+. The efflux of reducing compounds was determined on day 8 in pots containing 500 ml of solution. Immediately prior to assay, roots of intact plants were rinsed for 30 min in aerated 50 μM $CaSO_4$. Secretion of reductants into aerated 'efflux' solutions was then measured over two consecutive 4-h periods. The efflux solutions had a pH of 5.0 and contained 50 μM free Ca^{2+} (added as $CaSO_4$) and either 10 mM Mes/Na^+ or 10 mM acetic acid/Na^+. At intervals, 4.0 ml samples of efflux solution were removed and added to 4.0-ml aliquots of solution containing 80 μM $FeCl_3$, 200 μM TPTZ, 50 mM acetic acid/Na^+ (pH 3.9). After incubation in the dark for 24 h, optical densities were measured in a 1-cm cuvette at 593 nm. Concentrations of Fe^{III} were calculated assuming a molar absorptivity for $[Fe^{II}(TPTZ)_2]^{2+}$ of 22,600 (6).

Chemical Analysis of Tissues. Shoots were freeze dried and finely ground. Samples of this material were analyzed for Chl (*a* + *b*) as previously described (4). Root and shoot samples were dry ashed at 450°C and resuspended in 1% (w/v) HCl. Concentrations of Fe in shoot digests and K in root digests were determined by atomic absorption spectroscopy and flame photometry, respectively.

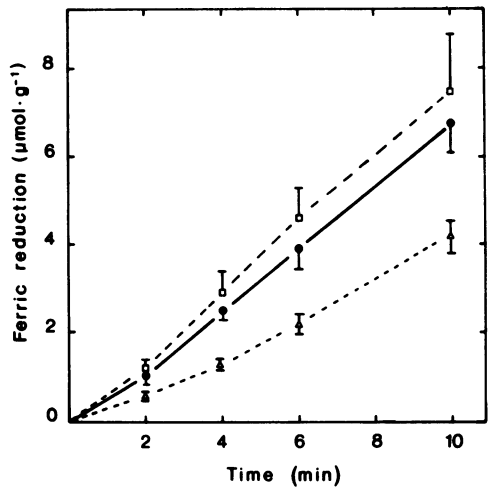

FIG. 1. Effect on rates of Fe^{III} reduction of exposing roots of low Fe peanuts to air or N_2. Immediately prior to assay rinsed roots of intact peanuts were incubated for 10 min in 50 μM $CaSO_4$ (●) or were exposed to air (□) or N_2 (△). Mean rates of Fe^{III} reduction (μmol Fe^{III} g^{-1} dry weight h^{-1}) were: roots incubated in $CaSO_4$ solution, 40.6; roots exposed to air, 44.9; roots exposed to N_2, 25.3.

Table I. *Effect of Presence and Subsequent Removal of Acetic Acid on Rates of K^+ Uptake by Roots of Peanuts*

Roots of low Fe peanuts were incubated for 30 min solutions containing either 10 mM Mes or 10 mM acetic acid at pH 5.0. Solutions also contained 0.5 mM K^+ labeled with ^{86}Rb, 7.0 mM Na^+, and 50 μM free Ca^{2+}. In some cases, roots had a pretreatment of 30 min in solutions of similar composition but without ^{86}Rb.

Pretreatment	Treatment	K^+ (^{86}Rb) Uptake
		$\mu mol\ g^{-1}\ dry\ wt\ h^{-1}$
	Mes	37.0
	Acetic acid	22.6
Mes	Mes	34.6
Acetic acid	Acetic acid	12.4
Acetic acid	Mes	34.4

Table II. *Effects of Mes and Acetic Acid in the Assay Medium on Rates of Fe^{III} Reduction by Roots of Intact Plants*

Assays for Fe^{III} reduction contained either 10 mM Mes/Na^+ or 10 mM acetic acid/Na^+ at pH 5.0. Each value is the mean ± SE of four (peanuts) or six replicates (sunflowers, maize).

Plant	High Fe Plants (Mes)	Low Fe Plants	
		Mes	Acetic acid
	μmol Fe^{III} reduced g^{-1} dry wt h^{-1}		
Peanuts	4.9 ± 1.8	27.3 ± 2.2	11.1 ± 1.4
Sunflowers	3.9 ± 0.5	7.3 ± 2.4	1.3 ± 0.3
Maize	0.29 ± 0.02	0.08 ± 0.01	0.08 ± 0.01

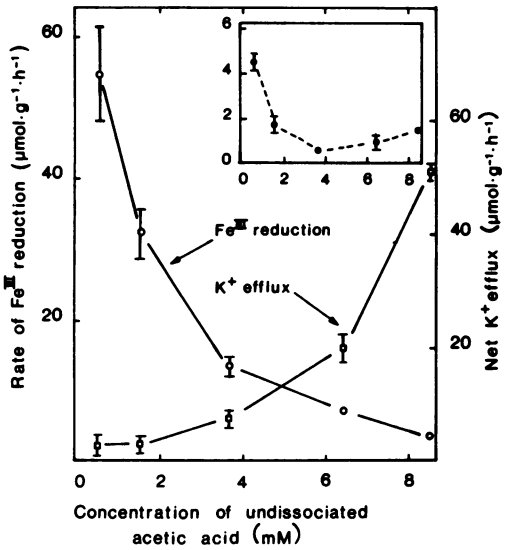

FIG. 2. Effect of acetic acid on rates of Fe^{III} reduction (○) and net K^+ efflux (□) by roots of low Fe peanuts. The inset shows the Fe^{III} reduction by high Fe plants (●). Assays contained 50 μM free Ca^{2+} (added as $CaCl_2$) and both 10 mM Mes/Na^+ and 10 mM acetic acid/Na^+ at pH values between 4.0 and 6.0. Each point is the mean ± SE of four replicates. Concentrations of K^+ (mmol g^{-1} dry weight) in the roots were 1.18 ± 0.01 (SE).

RESULTS

Growth of Plants. Dry weights of peanuts, sunflowers, and maize were not greatly affected by growth under conditions of low Fe. However, Chl concentrations (mg g^{-1} dry weight) decreased in low Fe plants (from 4.9 to 2.2 in peanuts; from 7.8 to 4.4 in sunflowers; from 6.0 to 3.7 in maize). Concentrations of Fe in the shoots (μg g^{-1} dry weight) also decreased in low Fe plants (from 190 to 72 in peanuts; from 193 to 53 in sunflowers; from 95 to 67 in maize).

Secretion of Reductants into the Free Space and Fe^{III} Reduction. An experiment was conducted with low Fe peanuts to determine if secreted compounds which reduce Fe^{III} accumulate in the free space when roots are removed from nutrient solution. If this was so, we would expect that subsequent replacement of roots in solutions containing Fe^{III}EDTA and BPDS would cause a 'pulse' of reduction as reductants accumulated in the free space came into contact with Fe^{III}.

It was found that the amounts of Fe^{III} reduced in assays increased linearly over a 10-min period not only with control roots incubated in solutions of 50 μM $CaSO_4$, but also with roots which had been previously exposed to air or N_2 for 10 min (Fig. 1). Rates of Fe^{III} reduction by roots exposed to air were comparable to rates of reduction by the control roots; however, Fe^{III} reduction by roots exposed to N_2 was 40% slower than Fe^{III} reduction by control roots (caption Fig. 1). The inhibitory effect on Fe^{III} reduction of a 10-min period of anoxia was confirmed in two other experiments (results not reported here).

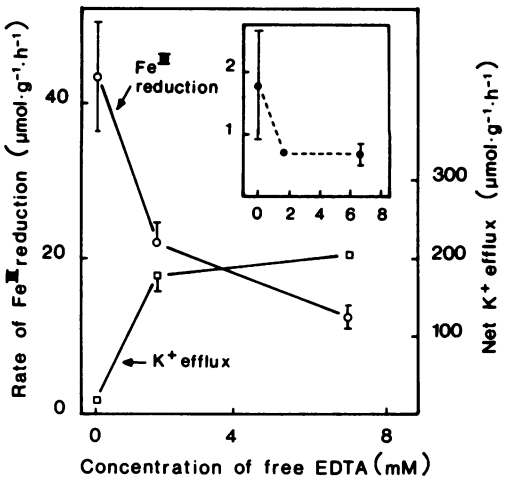

FIG. 3. Effect of free EDTA on rates of Fe^{III} reduction (○) and net K^+ efflux (□) by roots of low Fe peanuts. The inset shows Fe^{III} reduction by high Fe plants (●). Assays contained 7.5 mM EDTA (disodium salt) and various concentrations (0.9–8.4 mM) of $CaSO_4$. In this way, concentrations of free EDTA were varied from 0 to 6.6 mM, and assays containing zero free EDTA contained an excess of 50 μM free Ca^{2+}. Each point is the mean ± SE of four (low Fe plants) or three replicates (high Fe plants). Concentrations of K^+ (mmol g^{-1} dry weight) in the roots were 1.03 ± 0.02 (SE).

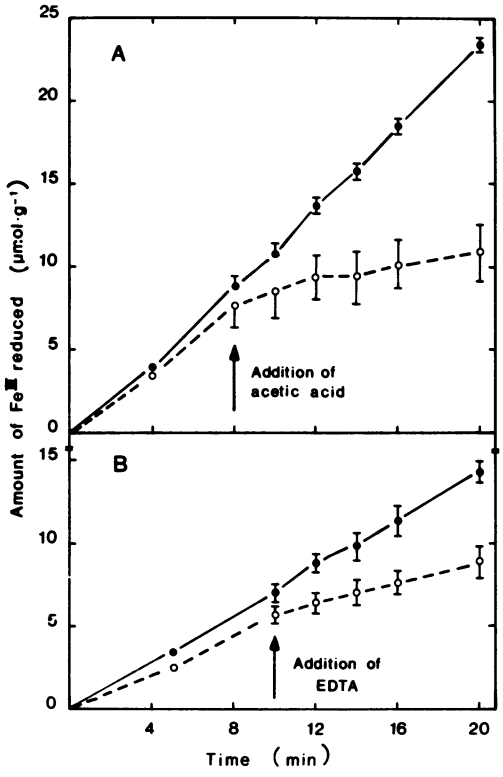

FIG. 4. Short term changes in the reduction of Fe^{III} by roots of low Fe peanuts after the addition (arrow) of (A) 10 mM acetic acid/Na^+ (pH 5.0) or (B) 2 mM EDTA (disodium salt). (●), Control assays containing 10 mM Mes/Na^+ (pH 5.0); (○), assays containing acetic acid/Na^+ or free EDTA after arrow. Each point is the mean ± SE of three (A) or four replicates (B).

Effects of Acetic Acid and EDTA on Membrane Integrity and

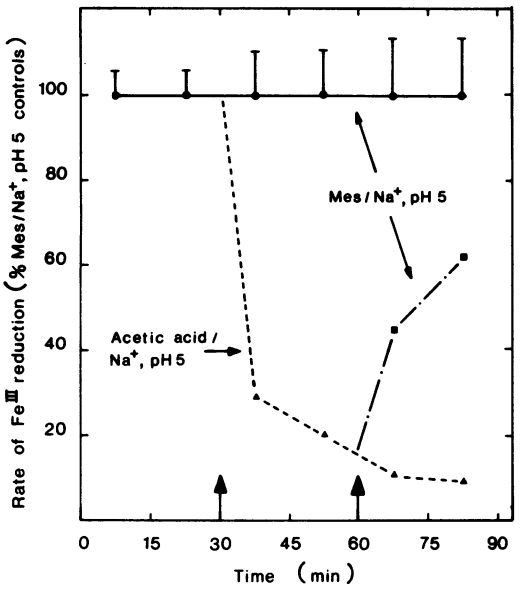

FIG. 5. Effect of the presence and subsequent removal of acetic acid on rates of Fe^{III} reduction by roots of low Fe peanuts. Solutions were changed at 30-min intervals (arrowed). (●), 10 mM Mes/Na^+ (pH 5.0) controls; (▲), plants treated with 10 mM Mes/Na^+ (pH 5.0) for 30 min, then 10 mM acetic acid/Na^+ (pH 5.0); (■), plants treated with 10 mM Mes/Na^+ (pH 5.0) for 30 min, then 10 mM acetic acid/Na^+ (pH 5.0) for 30 min, then 10 mM Mes/Na^+ (pH 5.0). Each point is the mean of 4, 8, or 12 replicates. SE indicated by vertical bars. Mean rates of Fe^{III} reduction (μmol g^{-1} dry weight h^{-1}) by the Mes/Na^+ (pH 5.0) controls were: 0 to 30 min, 29.3; 30 to 60 min, 27.0; 60 to 90 min, 24.6.

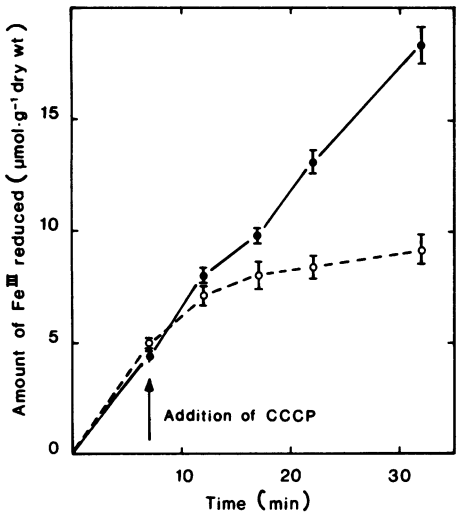

FIG. 6. Short term changes in the reduction of Fe^{III} by roots of low Fe peanuts after the addition (arrow) of CCCP (5 μM). (●), Control assays; (○), assays containing CCCP after arrow. Each point is the mean ± SE of four replicates.

Fe^{III} Reduction. The damaging effects of acetic acid on the integrity of plasma membranes were apparent in the inhibition of K^+ (^{86}Rb) uptake (Table I). Low Fe peanuts took up K^+ from solutions buffered with 10 mM Mes/Na^+ (pH 5.0) at about 35 μmol g^{-1} dry weight h^{-1}. However, rates of K^+ uptake were slower in solutions buffered with 10 mM acetic acid/Na^+ (pH 5.0). In an initial 30-min interval, rates of K^+ uptake were 40% slower in solutions with acetic acid than with Mes: in a subsequent 30-min interval, rates of K^+ uptake were 65% slower with acetic acid than with Mes (Table I). Replacement of solutions containing acetic acid by solutions buffered with Mes restored control levels of K^+ uptake (Table I).

Table III. *Effect of Acetic Acid on Efflux of Reductants by Roots of Peanuts*

Efflux of reductants was measured over two 4-h intervals. During the initial interval (0–4 h), roots were incubated in solutions containing 10 mM Mes/Na^+ (pH 5.0). During the second interval (4–8 h), roots were incubated in solutions which contained either 10 mM Mes/Na^+ or 10 mM acetic acid/Na^+ at pH 5.0. Reductants were assayed by incubating samples of nutrient solution with inorganic Fe^{III} and TPTZ for 24 h. Each value is the mean ± SE of seven replicates.

Treatment	Efflux of Reductants	
	0–4 h	4–8 h
	μmol g^{-1} dry wt h^{-1}	
Mes (0–4 h), Mes (4–8 h)	0.20 ± 0.06	0.25 ± 0.09
Mes (0–4 h), acetic acid (4–8 h)	0.18 ± 0.05	0.84 ± 0.03

The effect of acetic acid on Fe^{III} reduction was tested with peanuts, sunflowers, and maize. Fe^{III} reduction by roots was measured in solutions containing either 10 mM Mes/Na^+ or 10 mM acetic acid/Na^+ at pH 5.0 (Table II). With Mes, conditions of low Fe in sunflowers and peanuts (Fe-efficient species) caused 2- and 5-fold increases, respectively, in rates of Fe^{III} reduction; however, low Fe conditions in maize (an Fe-inefficient species) did not result in any increase in rates of Fe^{III} reduction. Rates of Fe^{III} reduction by low Fe peanuts and sunflowers decreased with acetic acid to 40% and 20% of the respective rates with Mes. In contrast, acetic acid had no effect on rates of Fe^{III} reduction by roots of low Fe maize (Table II).

The inhibitory effect of acetic acid on Fe^{III} reduction by Fe-efficient species was studied in more detail with peanuts. In one experiment, assay solutions contained both 10 mM Mes and 10 mM acetic acid adjusted with NaOH to pH values between 4.0 and 6.0. The net efflux of K^+ over a 30-min period from the roots into K^+ free solutions was used as a marker of the integrity of root plasma membranes. Close correlations were observed between the concentration of the undissociated acetic acid in the buffers, the rate of Fe^{III} reduction by roots of low Fe peanuts, and the net efflux of K^+ (Fig. 2). As concentrations of undissociated acetic acid in the external solution increased 17-fold from 0.54 mM (pH 6.0) to 8.52 mM (pH 4.0), rates of net K^+ efflux increased 19-fold, and rates of Fe^{III} reduction decreased by 94% (Fig. 2). High concentrations of undissociated acetic acid also inhibited Fe^{III} reduction by roots of high Fe peanuts (Fig. 2, inset). It is unlikely that the decreases in Fe^{III} reduction in this experiment were due to decreasing pH in the external solution; it is shown elsewhere that, in the absence of acetic acid, rates of Fe^{III} reduction by roots of Fe-deficient peanuts increase as the pH of the external solution falls from 6.0 to 5.0 (23).

Effects of membrane integrity on Fe^{III} reduction were also examined using the chelating agent EDTA. In one such experiment with roots of low Fe peanuts, net K^+ efflux increased 15-fold, and rates of Fe^{III} reduction decreased by 71% as the concentration of free EDTA increased from 0 to 6.6 mM (Fig. 3). EDTA also inhibited the Fe^{III} reduction by roots of high Fe peanuts (Fig. 3, inset).

The effects of acetic acid and EDTA on Fe^{III} reduction occurred very rapidly. In assays at pH 5.0 with low Fe peanuts, rates of Fe^{III} reduction decreased within 2 min of the addition of 10 mM acetic acid/Na^+ (pH 5.0), or 2 mM EDTA (disodium salt) (Fig. 4).

The inhibitory effects at pH 5.0 of 10 mM acetic acid/Na^+ were at least partially overcome by substituting these solutions for 10 mM Mes/Na^+ at pH 5.0: 15 to 30 min after this substitu-

tion, rates of Fe^{III} reduction had increased (2-fold) to 60% of the rates of control plants incubated throughout with Mes (Fig. 5).

Effect of CCCP on Fe^{III} Reduction. Fe^{III} reduction was also affected by the uncoupler CCCP. Rates of Fe^{III} reduction by roots of low Fe peanuts decreased within 5 min of the addition of CCCP (5 μM) to the assays (Fig. 6).

Effect of Acetic Acid on the Secretion of Reducing Compounds. An experiment was conducted with low Fe peanuts to determine the effects of acetic acid on the secretion of compounds which reduce Fe^{III}. This experiment was conducted over two consecutive 4-h time intervals. During the initial 4-h period, reductants were secreted into a 10 mM Mes/Na^+ (pH 5.0) solution at a rate of about 0.20 μmol g^{-1} dry weight h^{-1} (Table III). This rate of secretion of reducing compounds was less than 1% of the rate of Fe^{III} reduction by intact roots (*cf.* Table III with Table II). After 4 h, the Mes solutions were either renewed or replaced by 10 mM acetic acid/Na^+ (pH 5.0) buffer. Renewal of the Mes solutions had no effect on the rates of secretion of reductants. However, with acetic acid solutions there was a 4-fold increase in rates of secretion of reductants by the roots (Table III).

DISCUSSION

Membrane Integrity and Fe^{III} Reduction. Previous workers have established that EDTA and low mol wt organic acids rapidly inhibit ion uptake (9–11, 15) and increases the net efflux from roots of inorganic and organic cellular constituents (10, 13, 15, 16). In cases with low mol wt organic acids, these changes increase with the concentration of the undissociated acid (16) and are reversed by the transfer of roots into acid free solutions (10, 11).

In the experiments presented here with acetic acid and EDTA, the correspondence between increased net K^+ efflux from the roots and decreased rates of Fe^{III} reduction strongly suggests that plasma membrane integrity plays an important role in the enhanced Fe^{III} reduction by roots of low Fe peanuts (*cf.* Figs. 2 and 3). The inhibition of Fe^{III} reduction by acetic acid was partially reversed by substituting the acetic acid with Mes (Fig. 5). An inhibition of K^+(^{86}Rb) uptake due to treatment with acetic acid was also reversed by replacing acetic acid with Mes (Table I).

It is likely that the inhibition of Fe^{III} reduction by anoxia and CCCP (Figs. 1 and 6) is also due to adverse effects on plasma membrane integrity, and not ATP supply. Previous workers have shown that anoxia rapidly (within 15 min) causes the net efflux from roots of organic acids, amino acids, and K^+ (14), and that CCCP increases the permeability of membranes to H^+ before decreasing endogenous ATP concentrations (8).

Mechanism of Fe^{III} Reduction. It is difficult to reconcile the results of the present paper with the Olsen model (19, 20) of Fe^{III} reduction (*i.e.* reduction in rhizosphere by phenolics secreted from roots). In low Fe peanuts, the efflux of reducing compounds into external solutions was more than 100-fold slower than rates of Fe^{III} reduction by intact roots (*cf.* Tables II and III). Olsen *et al.* (20) account for this difficulty by suggesting that released reductants are oxidized enzymically in the presence of O_2. However, we were not able to demonstrate release of reducing compounds into the free space of roots, even under conditions of anoxia (Fig. 1). Furthermore, roots exposed for 10 min to N_2 reduced Fe^{III} at only 40% of the rate of control roots (caption Fig. 1).

If Fe^{III} reduction were due to the efflux of reducing compounds from the root, we might expect that damage to the root membranes could cause an increase in the release of reductants, leading to increased rates of Fe^{III} reduction. With low Fe peanuts, it was found that damage to the root plasma membranes using acetic acid did indeed promote the release of compounds capable of reducing inorganic Fe^{III} over a 24-h period (Table III); however, such damage did not increase the reduction of chelated Fe^{III} in the short term. On the contrary, Fe^{III} reduction decreased within 2 min of the exposure of roots to either acetic acid or EDTA (Fig. 4). Clearly, increased efflux of reductants is not necessarily associated with corresponding increases in Fe^{III} reduction.

The results of the present work are more consistent with the Bienfait/Chaney hypothesis (1, 5). If we accept the suggestion that Fe^{III} is reduced by a plasma membrane-bound enzyme, then damage to plasma membrane integrity might decrease rates of Fe^{III} reduction in two possible manners: (a) through decreased affinity of the Fe^{III} reductase for Fe^{III}; (b) through increased leakage from the epidermal cells of intracellular reductants and/or cofactors necessary for electron transfer to or from the enzyme.

In the present work, we argue that secreted reductants are not the electron donors for Fe^{III} reduction. This conclusion is also supported by other investigators (1, 5, 23). The question therefore remains as to the real function of secreted phenolics. We suggest that these compounds may play a role in the chelation of insoluble Fe^{III} in the rhizosphere which allows the diffusion of iron to the reduction sites at the plasma membranes of root epidermal and/or cortical cells.

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