Plant Physiol. (1987) 85, 310–314
0032-0889/87/85/0310/05/$01.00

Involvement of Superoxide Radical in Extracellular Ferric Reduction by Iron-Deficient Bean Roots[1]

Received for publication February 24, 1987 and in revised form May 29, 1987

ISMAIL CAKMAK[2], DIRK A. M. VAN DE WETERING, HORST MARSCHNER, AND H. FRITS BIENFAIT*
Institut für Pflanzenernährung, Universität Hohenheim, Postfach 70 05 62, Stuttgart 70, Federal Republic of Germany (I.C., H.M.); *Vakgroep Bodemkunde en Plantevoeding, Landbouwuniversiteit Wageningen, PB 8005, 6700 EC Wageningen, The Netherlands* (D.A.M.v.d.W.); *and Plantenfysiologisch Laboratorium, Universiteit van Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands* (H.F.B.)

ABSTRACT

The recent proposal of Tipton and Thowsen (Plant Physiol 79: 432–435) that iron-deficient plants reduce ferric chelates in cell walls by a system dependent on the leakage of malate from root cells was tested. Results are presented showing that this mechanism could not be responsible for the high rates of ferric reduction shown by roots of iron-deficient bean (*Phaseolus vulgaris* L. var Prélude) plants. The role of O_2 in the reduction of ferric chelates by roots of iron-deficient bean plants was also tested. The rate of Fe(III) reduction was the same in the presence and in the absence of O_2. However, in the presence of O_2 the reaction was partially inhibited by superoxide dismutase (SOD), which indicates a role for the superoxide radical, O_2^-, as a facultative intermediate electron carrier. The inhibition by SOD increased with substrate pH and with decrease in concentration of the ferrous scavenger bathophenanthrolinedisulfonate. The results are consistent with a mechanism for transmembrane electron transport in which a flavin or quinone is the final electron carrier in the plasma membrane. The results are discussed in relation to the ecological importance that O_2^- may have in the acquisition of ferric iron by dicotyledonous plants.

Dicotyledonous plants suffering from iron deficiency may develop so-called 'Fe efficiency reactions' such as rhizosphere acidification (23) and a strong increase in the capacity of the roots to reduce ferric chelates (Turbo reductase) (3, 4, 6, 22).

It was proposed that the Turbo reductase is located in the plasma membrane of the root epidermal cells (3, 26), and NADPH was proposed as the electron donor (27). It can reduce several kinds of ferric salts including ferricyanide (28). This inducible reducing system is therefore different from the Standard (basic) reducing system in the plasma membranes of monocotyledons and dicotyledons, which can donate electrons to ferricyanide but not to ferric chelates (3, 7, 10, 24). This latter activity is not influenced by the iron nutritional status of the plant; its function is still subject to speculation (17).

Analogous to iron deficient roots, lymphocytes which are exposed to infection develop an inducible electron transfer system in the plasma membrane which accepts electrons from cytosolic NADPH and transmits them to an extracellular acceptor. The natural electron acceptor is O_2 and the resultant O_2^- radicals are involved in killing pathogenic microorganisms (2).

The formation of O_2^- radicals by an NADPH oxidase at the plasma membrane is also a response phenomenon of plant cells to infection by pathogens (8, 9). Furthermore, plant cells catalyze an NAD(P)H-dependent O_2^- production during lignification (14, 15, 19). Also on the surface of intact pea roots, O_2^- formation is detectable (1).

Recently, Tipton and Thowsen (30, 31) proposed an alternative mechanism for ferric chelate reduction by roots of iron-deficient dicotyledons. In their model, malate accumulated in iron-deficient roots leaches out through the plasma membranes and reduces NAD^+ via cell-wall bound malate dehydrogenase. NADH would then reduce ferric chelates via an NADH-ferric chelate oxidoreductase in the cell walls. This model is very similar to that presented for the lignification process of plant cell walls (14).

In the present paper the proposal of Tipton and Thowsen (31) was tested. Additionally, the possible role of O_2^- in the extracellular ferric reduction was examined.

MATERIALS AND METHODS

French beans (*Phaseolus vulgaris* L. var Prélude, from Sluis, Enkhuizen, The Netherlands) were grown on modified Knop nutrient solution, with or without 40 or 100 μM Fe-EDTA as described elsewhere (4).

Ferric reduction activity was measured routinely in 5 mM Mes, 0.5 mM $CaSO_4$, 100 μM Fe-EDTA, and 300 μM BPDS[3] (pH 5.5) at 25°C, in a volume of 100 ml (intact root systems) or 2 ml (apical root zones and cell walls). The A_{540} was measured continuously by circulating the solution through a 2-cm cuvette of a Skalar photometer (Breda, The Netherlands). In experiments with cell walls, they were immobilized by a small plug of cotton wool. Anaerobic conditions in measurements with apical root zones were realized by addition of 10 mM glucose, 100 units glucose oxidase, and 1300 units catalase.

Cell walls of roots were prepared by milling fresh roots of 14-d-old plants in 1 mM Hepes-HCl (pH 7.6), 0.5 mM $CaSO_4$, 1% (w/g FW) polyvinylpolypyrrolidone, and 5% (w/g FW) sand, and repeated washing of the insoluble fraction with the extraction buffer by centrifugation (10 min at 5000*g* 0°C). After the first wash, the sand and the polyvinylpolypyrrolidone was separated from the precipitated root material.

[1] Supported by a grant from the Gesellschaft fur Technische Zusammenarbeit (GTZ) and the Bundesministerium fur Technische Zusammenarbeit (BMZ) to I. C.

[2] Present address: Department of Plant Nutrition, University of Cukurova, Adana, Turkey.

[3] Abbreviations: BPDS, bathophenanthrolinedisulfonate; FW, fresh weight; G6P dh, glucose 6-phosphate dehydrogenase; malate dh, malate dehydrogenase; SOD, superoxide dismutase.

G6P dh (EC 1.1.1.49) and malate dh (EC 1.1.1.37) activity in root fractions were determined in 1-ml incubations, continuously mixed on a slow rotator. At fixed intervals, a whole mixture was ejected through cotton wool into a cuvette, after which the absorption spectrum between 300 and 400 nm was quickly scanned. Reaction media were, for G6P dh, 20 mM Hepes, 1 mM glucose 6-P, 0.5 mM $NADP^+$, and 7.5 mM $MgCl_2$ (pH 7.6), and for malate dh, 20 mM Hepes, 0.12 mM NADH, and 0.8 mM oxaloacetate (pH 7.6). For determination of malate dh of isolated roots (apical 2–3 cm) they were placed in a Pasteur pipet closed at the tip, with their cut ends just above the meniscus.

SOD, glucose oxidase, and catalase were from Boehringer (Germany).

RESULTS

Role of Cell-Wall Bound Malate dh in Ferric Reduction. The amount of cell-wall bound malate dh in Fe-deficient bean roots was determined by homogenizing the roots with a low salt extraction medium (1 mM Hepes, 0.5 mM $CaSO_4$) and repeated washing of the 5000*g* precipitate, followed by determination of malate dh, and of G6P dh as a marker for cytosolic enzymes in the fractions. About 6% of the malate dh present in the crude homogenate remained in the cell wall fraction after repeated washing, while G6P dh in that fraction fell to less than 1% of the original level after the third washing (Table I). The amount of malate dh corresponded to an activity of 17 μmol NADH oxidized·h^{-1}·g^{-1} FW root tissue. Addition to the extraction buffer of 1 mM dithioerythrol, 1 mM EDTA, 10 mM Hepes, or various combinations of these, did not essentially affect the results; the malate dh activity remaining after washing was always less than 10% of the activity in the crude homogenate.

The activity of malate dh to reduce NAD^+ at pH 5.5 with 5 mM L-malate was 3.1% of the capacity to oxidize NADH. Thus, the amount of enzyme present in cell walls from 1 g FW root tissue could reduce 0.03 × 17 μmol or 0.5 μmol NAD^+·h^{-1}·g^{-1} FW, equivalent to 1.0 μmol Fe reduced·h^{-1}·g^{-1} FW. This is the rate which could be realized by malate dh in all the cell walls, *i.e.*, including the enzyme present in the stele and cortex. The ferric reduction activity is located in the epidermal and hypodermal cell layers (26) and therefore we measured also malate dh activity directly on isolated roots. Table II shows that oxaloacetate-depending NADH-oxidation at pH 7.6 was not faster than 4 μmol·h^{-1}·g^{-1} FW, regardless the iron nutritional status of the roots. The forward reaction, producing NADH with malate, could therefore not be more than 0.13 μmol·h^{-1}·g^{-1} FW, equivalent to 0.26 μmol Fe reduced·h^{-1}·g^{-1} FW. This value is about ¼ of that obtained with isolated cell walls.

Finally we tested the effect of added malate on the ferric reduction activity of Fe-sufficient and Fe-deficient roots. Table III shows that malate inhibited the ferric reducing activity of Fe-deficient roots by about 30%. Fe(III)-malate is reduced by Fe-deficient roots at 37% of the rate with Fe-EDTA (4); thus, the inhibition could have been caused by exchange of Fe(III) between 0.1 mM EDTA and 5 mM malate.

Table I. *Activities of G6P dh and Malate dh in Cell Wall Fractions from Fe-Deficient Bean Roots*

Fraction	G6P dh	Malate dh
	$\mu mol \cdot h^{-1} \cdot g^{-1}$ FW	
Crude extract	129	292
Precipitate:		
First	9	160
Second	0.8	18
Third		19
Fourth		11
Fifth		17

Table II. *Malate dh in the Free Space of Root Tips from Fe-Sufficient and Fe-Deficient Plants*

Isolated roots (apical 3 cm) were incubated in Pasteur pipets closed at the tip. The cut ends were kept above the meniscus in order to prevent contribution by cellular malate dehydrogenase.

Addition	NADH Oxidation	No. of Incubations
	$\mu mol \cdot h^{-1} \cdot g^{-1}$ FW ± SD	
None	0.0	2
Fe-sufficient roots	2.0	1
Fe-sufficient roots + 0.8 mM oxaloacetate	6.1 ± 2.0	3
Fe-deficient roots	1.7	1
Fe-deficient roots + 0.8 mM oxaloacetate	3.5 ± 0.2	3

Table III. *Effect of Malate and Glucose Additions on Fe-EDTA Reduction by Roots of Fe-Deficient and Fe-Sufficient Bean Plants*

Ferric reduction was measured in 100 ml 5 mM Mes, 0.5 mM $CaSO_4$, 100 μM Fe-EDTA, and 300 μM BPDS (pH 5.3, 25°C).

Preculture of Plants	Expt. No.	Ferric Reduction Activity		
		Control	+ 5 mM L-malate	+ 1 mM glucose
		$\mu mol \cdot h^{-1}$ g^{-1} FW		
−Fe	1	1.88	1.25	
	2	2.02	1.63	
	3	2.78		2.87 (after 2 h)
	4	2.67		2.90 (after 2 h)
+Fe	5	0.30	0.30	

NADH Oxidation by Cell Walls. NADH in cell walls might reduce ferric chelates directly, via an NADH-ferric oxidoreductase, as proposed by Tipton and Thowsen (31), or, indirectly, via reduction of oxygen to the superoxide radical, $O_2^{\cdot-}$, which then could reduce ferric chelates either enzymatically or nonenzymically (11). The results presented in Table II indicate that NADH was oxidized by roots of bean plants. The capacity of NADH to reduce Fe-EDTA in the presence of cell walls from Fe-deficient plants is shown in Figure 1. The effect of SOD illustrates the role of $O_2^{\cdot-}$ in the process, particularly at higher pH. The ferric reduction by NADH at pH 5.5, 1.0 μmol Fe(II)·h^{-1}·g^{-1} FW cell walls, corresponds to 0.5 μmol Fe(II)·h^{-1}·g^{-1} FW root tissue. As in the case with malate dh, this is a maximal value, because ferric chelate reduction only takes place in the outer cell layers. Figure 2A shows that isolated roots of Fe-sufficient plants catalyzed Fe-EDTA reduction when NADH was added. The rate was less than 0.2 μmol Fe(II)·h^{-1}·g^{-1} FW. The nearly complete inhibition by SOD indicates that the reduction is through $O_2^{\cdot-}$ formation. The ferric reduction capacity of Fe-deficient root tips was also increased by addition of NADH, more so than in roots of Fe-sufficient plants. This increase was also nullified by SOD (Fig. 2B).

Role of Superoxide Radical in the Ferric Reduction Capacity. The role of $O_2^{\cdot-}$ in the process of ferric reduction in Fe-deficient bean roots was tested with SOD. At pH 6.6, reduction of Fe-EDTA was not inhibited by anaerobiosis, at least during short (less than 30 min) incubations (Table IV). SOD had no effect in the absence of O_2; but in the presence of O_2 it inhibited by about 60%. BSA at the same protein concentration had no effect, neither had catalase or boiled SOD (no data shown). These results suggest a facultative role for $O_2^{\cdot-}$ in the ferric reduction mechanism. The inhibiting effect of SOD was maximal under condi-

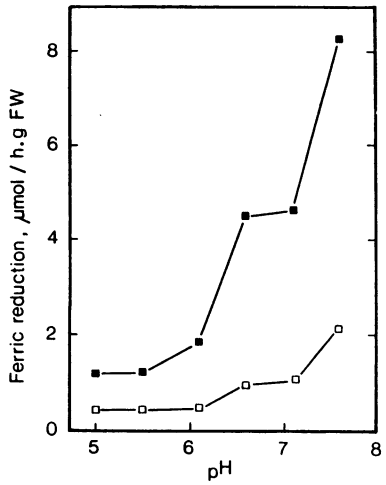

FIG. 1. Reduction of Fe-EDTA by NADH in the presence of cell walls from Fe-deficient bean roots (20 mg FW). Cell walls were incubated in 2 ml 5 mM Mes, 0.5 mM $CaSO_4$, 100 μM Fe(III)-EDTA, and 300 μM BPDS. The A_{540} was monitored continuously by pumping the solution through a spectrometer cuvette. Ferric reduction without (■) and with (□) addition of 250 units SOD.

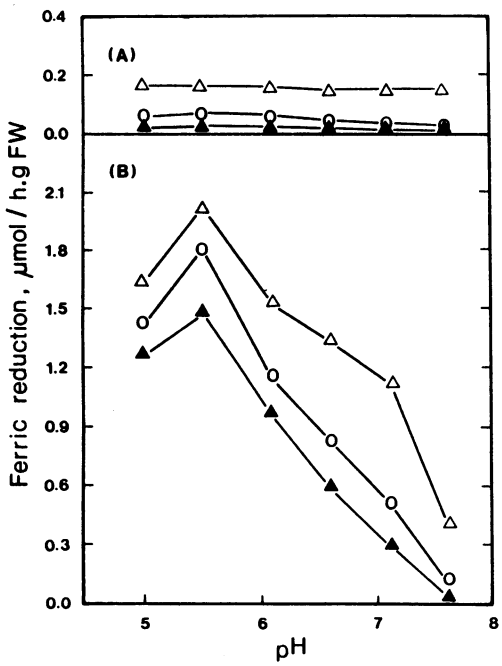

FIG. 2. Reduction of Fe-EDTA by bean roots (apical 3 cm). Roots of Fe-sufficient (A) and Fe-deficient (B) plants were incubated as described in Figure 1. O, no addition; △, after addition of 250 μM NADH; ▲, after addition of both 250 μM NADH and 250 units SOD.

tions where the chances for ferrous ions to be reoxidized by O_2, instead of being scavenged by BPDS, were highest: at low concentrations of BPDS (Fig. 3) and at high pH (Fig. 4). Interestingly, SOD also strongly inhibited at low Fe-EDTA concentrations (Table V). At high concentrations of Fe-EDTA and BPDS, inhibition by SOD reached a plateau of 30 to 35%, irrespective of pH between 5.5 and 7.1 (Table V, Fig. 3).

Apparently, under the conditions used by us between 30 (pH 5.5) and 75% (pH 7.1) of the ferric chelates that were reduced in the presence of O_2, received the electrons via O_2^- radicals which could be scavenged by SOD.

Table IV. *Effect of SOD on Aerobic and Anaerobic Reduction of Fe-EDTA by Isolated Roots of Fe-Deficient Bean Plants*

Incubation conditions were as described in the legend of Figure 1. Anaerobiosis was realized by addition of 10 mM glucose, glucose oxidase, and catalase at pH 7.1.

Addition	Ferric Reduction	
	$+O_2$	$-O_2$
	$\mu mol \cdot h^{-1} \cdot g^{-1}$ FW	
Control	2.6	2.9
250 units SOD	1.1	2.5

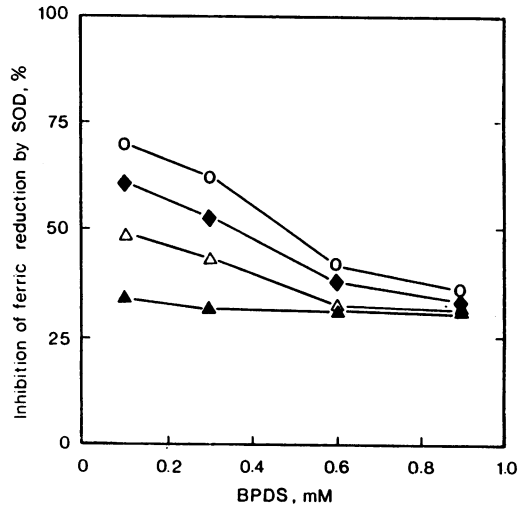

FIG. 3. Effect of the ferrous scavenger BPDS on the inhibition by SOD of Fe-EDTA reduction by iron-deficient bean roots (apical 3 cm). The conditions were as described in Figure 1. ▲, pH 5.5; △, pH 6.1; ♦, pH 6.6; ◇, pH 7.1.

DISCUSSION

Role of the 'Malate Route' in Ferric Reduction. The possibility was tested that roots of Fe-deficient bean plants reduce ferric chelates by a mechanism which involves leaching of malate from the root cells, reduction of NAD in the cell walls, and an NADH-ferric oxidoreductase in the cell walls, as proposed by Tipton and Thowsen (31).

Cell walls of Fe-deficient and Fe-sufficient plants contained a significant malate dehydrogenase activity (Table 1). The rate at which this enzyme could donate electrons to NAD^+, with 5 mM malate, was 0.26 μmol electrons·h^{-1}·$^1g^{-1}$ FW for the enzyme accessible to added malate in intact root tissue.

Apical root zones also oxidized added NADH; added NADH stimulated the reduction of Fe-EDTA (Fig. 2). This NADH-dependent ferric reduction was completely inhibited by superoxide dismutase. We assume therefore that NADH acts via formation of O_2^- radicals which in turn reduce Fe-EDTA (11). Oxidation of NADH in cell walls and the concomitant production of O_2^- has been documented, and a role in lignin biosynthesis is assumed (14, 15, 29). This is possibly the 'iron reductase' isolated by Tipton and Thowsen (31). The enzyme activity we found with apical root zones was less than 0.3 μmol Fe(II)·h^{-1}·g^{-1} FW.

From the foregoing it follows that the reduction of ferric chelates in the root cell walls through the combined action of malate dehydrogenase and NADH oxidase could attain a maximal rate of about 0.3 μmol Fe·h^{-1}·g^{-1} FW at pH 5.5. Indeed, the low rate of Fe-EDTA reduction by Fe-sufficient roots (0.3 μmol·h^{-1}·g^{-1} FW) was not visibly increased by addition of malate; ferric reduction by Fe-deficient roots was even slightly

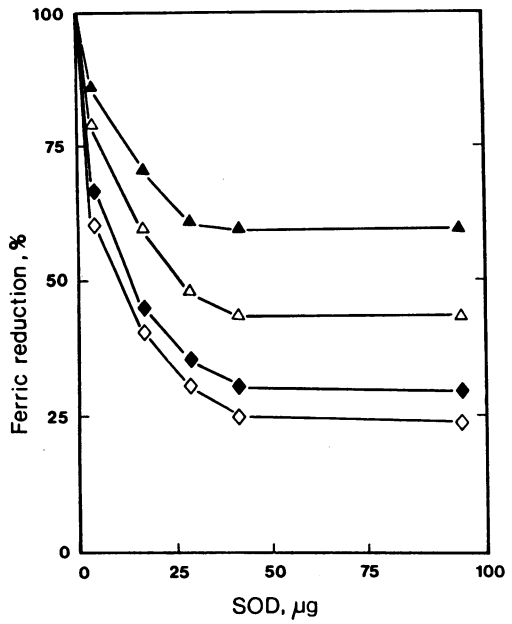

FIG. 4. Effect of SOD on Fe-EDTA reduction by Fe-deficient bean roots. The conditions were as described in Figure 1. ▲, pH 5.5; △, pH 6.1; ♦, pH 6.6; ◇, pH 7.1.

Table V. *Effect of SOD on Aerobic Reduction of Fe-EDTA by Isolated Bean Roots as a Function of Fe-EDTA Concentration at pH 6.1*

Conditions were as described in the legend of Figure 1. The BPDS concentration was 300 μM.

Fe-EDTA	Fe-EDTA Reduction Rate	Inhibition by SOD
μM	$\mu mol \cdot h^{-1} \cdot g^{-1}$ FW	%
100	1.03	40
150	2.13	30
200	2.81	27
250	3.38	26

inhibited.

The ferric chelate reduction activity of intact roots of Fe-deficient plants was about 2 $\mu mol \cdot h^{-1} \cdot g^{-1}$ FW (Table III) with 100 μM Fe-EDTA; at 300 μM Fe-EDTA the rate is near V_{max} and about 5 $\mu mol \cdot h^{-1} \cdot g^{-1}$ FW (4). The measured capacity and that of the 'malate route' differ therefore with a factor of at least 15.

Tipton and Thowsen (31) reported a strong stimulation of ferric reduction by added malate or glucose after prolonged incubation (5 h). We have similar results and assume that in prolonged incubations glucose and malate affect ferric reduction indirectly, by being taken up by the root cells and thereby influencing overall metabolism. This may lead to elevated levels of NADPH, increased potential fluxes of reducing equivalents to NADPH, or both.

We conclude that a pathway for electrons via malate leaching, NAD^+ reduction by malate and reoxidation by O_2, yielding O_2^- which reduces ferric chelates can only make a minor contribution to ferric reduction by iron-deficient bean roots at pH below 6. At high pH values, more favorable for the reduction of NAD^+ by malate, the 'malate route' might have a bigger share in the ferric reduction process.

Role of Superoxide Radicals in Ferric Reduction. The stimulation, by NADH, of Fe-EDTA reduction by isolated roots of Fe-deficient plants was more than 100% reversed by SOD (Fig. 2A). This indicated that also in ferric reduction which was independent of added NADH O_2^- participates. This was indeed the case (Fig. 4), although the rate of ferric reduction was the same in the presence or absence of O_2 (Table IV). Apparently, Fe-EDTA alone can get hold of all the available electrons in the absence of O_2; but in its presence, O_2 scavenges at least part of the electrons, and the O_2^- formed can then pass them to Fe(III) if SOD is not added. The reaction kinetics are complicated by the fact that ferrous ions may be reoxidized by O_2 and thus give rise to O_2^- (19). A high BPDS concentration is needed to chelate all the ferrous iron which is formed, but is also deleterious to the roots. By careful titration with BPDS and extrapolation of the curves obtained (Fig. 3) it became apparent that when Fe reoxidation was minimal, SOD inhibited about 30 to 35% at 100 μM Fe-EDTA in the pH range tested.

From results we obtained earlier we must conclude that O_2 cannot accept electrons from the induced 'Turbo' ferric chelate reducing system in the absence of added ferric chelate or ferricyanide: (a) The redox poise of NADP in aerobic roots of Fe-deficient plants was shifted to a more oxidized state within 2 min after addition of ferricyanide (27); (b) The potential difference over the plasma membrane of aerobic root epidermis cells was lowered only after addition of Fe-EDTA or ferricyanide (28).

A simple mechanism for ferric reduction by the Turbo system in bean roots, which accommodates the present and previous observations is the following (Fig. 5). In the plasma membrane of the Fe-deficient epidermal cell a two-electron carrier is present (XH_2). This carrier can donate an electron to Fe-EDTA or ferricyanide, but not to oxygen. After donation of one electron, $XH\cdot$ results. $XH\cdot$ can donate an electron to Fe-EDTA or ferricyanide but also to oxygen. After this reaction the resulting X can accept 2 electrons from cytosolic NADPH, and the system is regenerated. X might, most likely, be a flavin or a quinone (20).

Roots of many plant species excrete riboflavin when suffering from Fe shortage (32, 33). Under the same conditions microorganisms including the green alga *Chlorella* may synthesize flavoproteins called flavodoxins, believed to replace Fe-S proteins (18). Replacement of an Fe containing compound normally present in a plasma membrane redox chain, such as the Standard reductase (3), by a flavodoxin could change the properties of the redox system such that it can reduce extracellular ferric chelates. However, in flavodoxins the rate constant for the one-electron transfer from the fully reduced FH_2 to O_2 is characteristically higher than for the reaction with the halfreduced $FH\cdot$; the E_{m7} of the flavin group for the second electron transfer of known

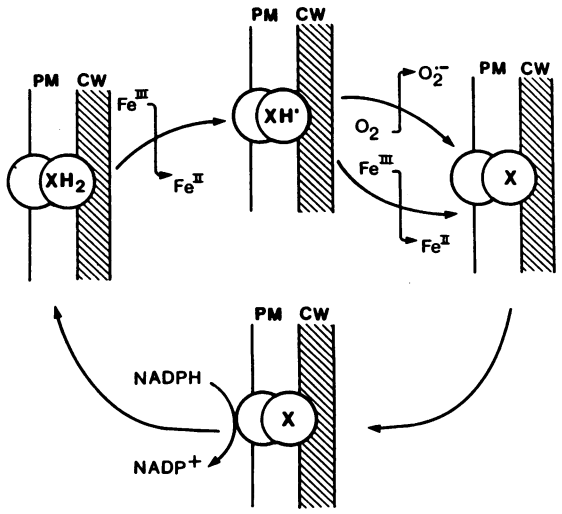

FIG. 5. Electron donation to ferric chelates and oxygen at the plasma membrane of epidermal cells in Fe-deficient bean roots. X is a two-electron carrier. PM, plasma membrane; CW, cell wall.

flavodoxins is at least 0.17 V more positive than for the first (18). This property is not in accordance with an increased capacity for $XH\cdot$ to reduce O_2 to $O_2^{\cdot-}$, as proposed in Figure 5.

In free flavins and flavin-containing oxidases the reaction of O_2 with the fully reduced compound is equally fast, yielding directly H_2O_2 (5). However, the half-reduced form of free flavin, once reduced, is more reactive with O_2 (13). A role for a flavin as X, in which all forms are more or less equally stabilized by protein binding, thus without changes in the E_{m7} values but with sufficiently lowered rate constants for oxidation by O_2, can therefore not be excluded.

The quinones are plausible candidates for the function of X. In contrast to flavins which are permanently fixed to a protein in each of the three redox states, quinones present in the redox states XH_2 and X diffuse freely in the membrane both in the lateral and the transmembrane direction. They are therefore ideally suited to transfer reducing equivalents, with or without protons, across the membrane. The values for E_{m7} of naturally occurring quinones vary between 0 and +0.1 V, suitable to reduce ferric-organic complexes such as Fe-EDTA (E_{m7} 0.12 V) (25). Assuming a moderate stabilization of the semiquinone, the one-electron redox couples $X\cdot/XH_2$ (first oxidized in Fig. 5) and $X/XH\cdot$ (second oxidized) will have values of E_{m7} that are about 0.1 V higher and lower, respectively, than the E_{m7} of the two-electron couple. This makes the semiquinone a better one-electron donor to oxygen than the fully reduced quinol (21, 34, 35). On the other hand, if the shift is not too strong, the fully reduced form will not be seriously hampered in its capacity to reduce ferric chelates.

The proposed mechanism differs from the one active in the plasma membrane of leucocytes. There, a one-electron carrier, Cyt b_{559}, receives electrons from NADPH via a flavin and a sulfide group, reducing O_2 to $O_2^{\cdot-}$ (12). A quinone is not involved (16).

In conclusion, the involvement of $O_2^{\cdot-}$ radicals in the electron transfer to extracellular ferric chelates might be of considerable importance for the acquisition of iron by roots of dicotyledonous species in substrates of low Fe availability, calcareous soils in particular. In contrast to the experimental conditions using relatively high concentrations of chelated Fe in combination with a scavenger (BPDS) for the reduced Fe(II), in Fe-deficient soils such scavengers are absent and the concentrations of chelated Fe(III) is very low. It has been shown in the present paper that the contribution of $O_2^{\cdot-}$ radicals to the overall process of ferric reduction is remarkably augmented by decreasing concentrations of chelated Fe(III) and BPDS, and also by increasing pH values.

Acknowledgments—We thank Dr. E. de Vries (Biochemical Laboratory, University of Amsterdam) for stimulating discussions, and Prof. Dr. E. F. Elstner (Botanisches Institut der Technischen Universität München) for valuable suggestions.

LITERATURE CITED

1. AVER'YANOV AA 1985 Superoxide radical generation by intact pea roots (Engl Transl). Sov Plant Physiol 32: 202–206
2. BADWEY JA, ML KARNOVSKY 1980 Active oxygen species and the functions of phagocytic leucocytes. Annu Rev Biochem 49: 695–726
3. BIENFAIT HF 1985 Regulated redox processes at the plasmalemma of plant root cells and their function in iron uptake. J Bioenerg Biomembr 17: 73–83
4. BIENFAIT HF, RJ BINO, AM VAN DER BLIEK, JF DUIVENVOORDEN, JM FONTAINE 1983 Characterization of ferric reducing activity in roots of Fe-deficient Phaseolus vulgaris. Physiol Plant 59: 196–202
5. BRIGHT HJ, DJT PORTER 1975 Flavoprotein oxidases, *In* PD Boyer, ed, The Enzymes, Ed 3, Vol XII, Academic Press, New York, pp 421–505
6. CHANEY RL, JC BROWN, LO TIFFIN 1972 Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiol 50: 208–213
7. CRAIG TA, FL CRANE 1981 Evidence for a *trans*-plasma membrane electron transfer system in plant cells. Proc Indiana Acad Sci 90: 150–155
8. DOKE N 1983 Generation of superoxide anion by potato tuber protoplasts during the hypersensitive response to hyphal wall components of Phytophtora infestans and specific inhibition of the reaction by suppressors of hypersensitivity. Physiol Plant Pathol 23: 359–367
9. DOKE N, HB CHAI 1985 Activation of superoxide generation and enhancement of resistance against compatible races of Phytophtora infestans in potato plants treated with digitonin. Physiol Plant Pathol 27: 323–334
10. FEDERICO S, CE GIARTOSIO 1983 A transplasmamembrane electron transport system in maize. Plant Physiol 73: 182–184
11. FONG KL, PB MCCAY, JL POYER 1976 Evidence for superoxide-dependent reduction of Fe(III) and its role in enzyme-generated hydroxyl radical formation. Chem Biol Interact 15: 77–89
12. GABIG TG, BA LEFKER 1986 Catalytic properties of the resolved flavoprotein and cytochrome B components of the NADPH dependent $O_2^{\cdot-}$ generating oxidase from human neutrophils. Biochem Biophys Res Commun 118: 430–436
13. GIBSON QH, JW HASTINGS 1962 The oxidation of reduced flavin mononucleotide by molecular oxygen. Biochem J 83: 368–377
14. GROSS GG, C JANSE, EF ELSTNER 1977 Involvement of malate, monophenols, and the superoxide radical in hydrogen peroxide formation by isolated cell walls from horseradish (*Armoracia lapathifolia* Gilib). Planta 136: 271–276
15. HALLIWELL B 1978 Lignin synthesis: the generation of hydrogen peroxide and superoxide by horseradish peroxidase and its stimulation by manganese (II) and phenols. Planta 140: 81–88
16. LUTTER R, R VAN ZWIETEN, RS WEENING, MN HAMERS, D ROOS 1984 Cytochrome *b*, flavins, and ubiquinone-50 in enucleated human neutrophils (polymorphonuclear leucocyte cytoplasts). J Biol Chem 259: 9603–9606
17. LÜTTGE U, DT CLARKSON 1985 Mineral nutrition: plasmalemma and tonoplast redox activities. *In* HD Behnke, K Esser, K Kubitzki, M Runge, H Ziegler, eds, Progress in Botany, Vol 47. Springer-Verlag, Berlin, pp 73–86
18. MAYHEW SG, ML LUDWIG 1975 Flavodoxins & electron transferring flavoproteins. *In* PD Boyer, ed, The Enzymes, Ed 3, Vol XII. Academic Press, New York, pp 57–118
19. MICHELSON AM 1977 Production of superoxide by metal ions. In AM Michelson, JM McCord, I Fridovich, eds, Superoxide and Superoxide Dismutases. Academic Press, New York, pp 77–86
20. MISRA HP, I FRIDOVICH 1972 The univalent reduction of oxygen by reduced flavins and quinones. J Biol Chem 247: 188–192
21. RICH PR, DS BENDALL 1980 The kinetics and thermodynamics of the reduction of cytochrome *c* by substituted *p*-benzoquinols in solution. Biochim Biophys Acta 592: 506–518
22. RÖMHELD V, H MARSCHNER 1983 Mechanism of iron uptake by peanut plants. I. Fe(III) reduction, chelate splitting and release of phenolics. Plant Physiol 71: 949–954
23. RÖMHELD V, C MÜLLER, H MARSCHNER 1984 Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiol 76: 603–606
24. RUBINSTEIN B, AI STERN, RG STOUT 1984 Redox activity of oat root cells. Plant Physiol 76: 386–391
25. SCHWARZENBACH G, J HELLE 1951 Die Eisen(II) und Eisen(III)-komplexone der Äthylendiamin-tetraessigsaure und ihr Redoxgleichgewicht. Helv Chim Acta 34: 576–591
26. SIJMONS PC, W VAN DEN BRIEL, HF BIENFAIT 1984 Cytosolic NADPH is the electron donor for extracellular FeIII reduction in iron-deficient bean roots. Plant Physiol 75: 219–221
27. SIJMONS PC, HF BIENFAIT 1983 Source of electrons for extracellular Fe(III) reduction in iron-deficient bean roots. Plant Physiol 59: 409–415
28. SIJMONS PC, FC LANFERMEIJER, AH DE BOER, HBA PRINS, HF BIENFAIT 1984 Depolarization of cell membrane potential during trans-plasma membrane electron transfer to extracellular electron acceptors in iron-deficient roots of *Phaseolus vulgaris* L. Plant Physiol 76: 943–946
29. STICH K, R EBERMANN 1984 Investigation of hydrogen peroxide formation in plants. Phytochemistry 23: 2719–2722
30. TIPTON CL, J THOWSEN 1983 Reduction of iron by soybean roots: correlation with iron efficiency on calcareous soils. Iowa State J Res 57: 409–422
31. TIPTON CL, J THOWSEN 1985 Fe(III) reduction in cell walls of soybean roots. Plant Physiol 79: 432–435
32. VENKAT-RAJU K, H MARSCHNER, V RÖMHELD 1972 Effect of iron nutritional status on ion uptake, substrate pH and production and release of organic acids and riboflavin by sunflower plants. Z Pflanzenernaehr Bodenkd 132: 177–190
33. WELKIE GW, GW MILLER 1960 Iron nutrition of *Nicotiana tabacum* L. in relation to riboflavin, riboflavin-5-phosphate, and flavin adenine dinucleotide content. Plant Physiol 36: 516–520
34. WOOD PM 1974 The redox potential of the system oxygen-superoxide. FEBS Lett 44: 22–24
35. YAGUZHINSKI LS, LI BOGUSLAVSKI, AD ISMAILOV 1974 Potential generation in bilayer lipid membranes in the NADH-flavin mononucleotide-ubiquinone-6-O system. Biochim Biophys Acta 368: 22–28