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SHORT REVIEW

Regulated Redox Processes at the Plasmalemma of Plant Root Cells and Their Function in Iron Uptake

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Abstract

Plants take up iron as ferric chelates or, after reduction, as ferrous ions. Ferric reduction takes place at the plasma membrane of the root epidermis cells by a transmembrane redox system, which can be activated when iron is getting short. It is proposed that this inducible system, with NADPH as electron donor, is separate from a system, presumably present in all plant cells, which transports electrons from NADH or NADPH to ferricyanide, or, *in vivo*, oygen.

Key Words: Plasma membrane; NADH oxidase; NADPH oxidase; roots; iron uptake; ferric reductase; transfer cell; chlorosis.

Introduction

The regulation of iron uptake and transport in plants has been studied for a long time, but the mechanisms involved are still largely unknown.

Most of the iron taken up by plants is built into the systems for electron transport, as in photosynthesis and respiration. Thus, it has been estimated that 80% of leaf iron is located in the chloroplasts (Liebich, 1941; Terry and Low, 1982). However, an excessive supply of iron to the leaves is undesirable; overdoses may cause the leaf cells and, eventually, the whole plant to die. This "iron toxicity" is commonly found in flooded fields, where oxygen cannot readily diffuse into the soil: microorganisms deplete the oxygen, turn to other electron acceptors and, in doing so, create conditions around the roots whereby iron is largely present in the ferrous form, which is easily taken up by the roots (Foy *et al.*, 1978). The mechanism by which an iron overload exerts its damaging effects probably is the same as, or similar, to that in

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mammals. Plants, like animals and microorganisms, have at their disposal the inducible iron storage protein ferritin (Hyde *et al.*, 1962; Seckbach, 1972; Bienfait and van der Mark, 1983), but, as in humans, its synthesis and storage capacity have limits. On the other hand, a shortage of iron leads to the decreased synthesis of iron-containing proteins and, most conspicuously, to the decreased production of chlorophyll. The resulting chlorophyll deficiency leads directly to lowered capacity of the photosynthesis system. It is therefore imperative for plants to maintain a well-regulated supply of iron.

Soils may contain any amount of iron; when aerated, it occurs mostly in the ferric form, as oxides or hydroxides. The free ferric ion concentration normally is very low: the solubility constant of $Fe(OH)_3$ is $10^{-38.7}$ (Biedermann and Schindler, 1957). For normal plant growth it has been estimated that the concentration of available ferric ion in the soil solution should be minimally on the order of 10^{-7} M (Lindsay and Schwab, 1982). In the presence of organic matter, the ferric ion may be solubilized by all kinds of chelators. Among those that are especially interesting are the specific iron chelators which are excreted by microorganisms (siderophores) (Stutz, 1964; Powell *et al.*, 1982; Cline *et al.*, 1983). These are mainly hydroxamates, such as ferrioxamine (Bickel *et al.*, 1960; Szaniszlo *et al.*, 1981; Powell *et al.*, 1982), and phenolics such as enterochelin (O'Brien and Gibson, 1970). Recently, it has become clear that the root itself may produce ferric-chelating compounds. Examples are mucigeneic acid (Mino *et al.*, 1983) and avenic acid (Fushiya *et al.*, 1980), both structurally related to the nonprotein amino acid nicotianamine, a siderophore first isolated from tobacco leaves (Noma *et al.*, 1971) which is thought to be an iron carrier in the plant itself (Scholz, 1970; Budesinsky *et al.*, 1980; Ripperger and Schreiber, 1982). Curiously, the related siderophores have, until now, only been found in exudates from monocotyledons. Caffeic acid is excreted as a siderophore upon iron starvation by tomato plants (Olsen *et al.*, 1981b).

The Uptake System for Iron

In aerobic soils, where ferric ion is the predominant form, iron chelates may be taken up as such by the young root parts. This process is rather slow but, depending upon the need of the plant on the one hand, and the concentration of the ferric chelate and the flow rate of the soil solution to the roots on the other hand, this process may be prevalent. Grasses, which have been found to excrete specific ferric chelators upon iron starvation, as mentioned above, may depend exclusively on this system of iron uptake.

Dicotyledonous species, such as beans, can reduce ferric chelates at the surface of the young roots; the resulting ferrous ion can be taken up readily.

If a plant suffers from iron shortage, the reductive system is strongly activated. Moreover, within a few hours the roots may lower the pH in the soil solution to values of 3 or even lower (Oertli and Jacobson, 1960; Marschner *et al.*, 1974; Brown 1978).

Decrease of pH

The capacity of a plant to lower the pH on the outside of the root is not extraordinary in itself. Excretion of protons in exchange for K^+ and NH_4^+ ions that are taken up is a well-known phenomenon. Thus, one way to cure an iron shortage in the field is to give large amounts of ammonium or potassium salts, so that the pH around roots is lowered and iron salts may be more easily solubilized (Barak and Chen, 1983). However, the pH decrease which is induced by iron shortage occurs under conditions where iron-sufficient plants raise the pH, mainly as a consequence of nitrate uptake, which is exchanged for OH^-. Thus a mechanism exists by which iron starvation causes the roots to expel protons at high rates. Recent work by Marschner's group indicates that it is the cause of the fastest known process of proton extrusion by root cells. The order for agents which increase proton release is Fe shortage > NH_4^+ > K^+ > fusicoccin (Römheld, *et al.*, 1984). The capacity of iron-stressed plants to lower the pH in the rhizosphere is not a general phenomenon; e.g., no grasses have yet been found that show the response.

Ferric Reduction

The roots of dicotyledonous plants and some monocots reduce ferric chelates at appreciable rates. Upon the development of iron deficiency, this reduction capacity increases gradually, e.g., from 0.5 μmol Fe(III) reduced$\cdot h^{-1}\cdot$g FW^{-1} to 5 μmol Fe(III)$\cdot h^{-1}\cdot$g FW^{-1} with Fe-EDTA (Bienfait *et al.*, 1982a). The substrate specificity is low (Bienfait *et al.*, 1983), as may be expected, for in the field the root may be confronted with any kind of ferric chelate. Two microbial chelates, ferrioxamine B and ferric aerobactin, are not reduced (Bienfait *et al.*, 1983). Ferricyanide seems to be the best substrate in terms of rate (Brown *et al.*, 1961; Bienfait, unpublished); DCIP and phenazine methosulfate are also reduced (Sijmons and Bienfait, 1983). Curiously, ferricyanide is also reduced by maize roots (Federico and Giartosio, 1983), but ferric chelates are not (Brown, 1978). Thus, it seems that the inducible system in dicots is characterized by its ability to reduce ferric chelates with organic acids. It is highly improbable that the free ferric

ion is reduced (Chaney *et al.*, 1972; Römheld and Marschner, 1983b; Bienfait *et al.*, 1983).

The mechanism of ferric reduction has been the subject of some controversy. After Brown and his colleagues in Beltsville had shown the existence and the inducibility of the ferric reduction system (Brown *et al.*, 1961), they demonstrated that the reduction occurred outside the root cell (Chaney *et al.*, 1972). They postulated that an enzyme system in the plasmalemma was capable of transferring electrons to the ferric chelates outside. Later, they stressed the role of reducing compounds which are excreted by the roots of iron-deficient plants, especially when the pH is lowered and the roots apparently become leaky (Brown and Ambler, 1973; Brown and Jones, 1974b; Olsen and Brown, 1980; Olsen *et al.*, 1981a, b; Olsen *et al.* 1982). As the group of Brown focused on the characterization of these "reductants," the process occurring directly at the cell surface was largely neglected. It is plausible that excreted reducing compounds play a role in chelating ferric ions in the rhizosphere (Olsen *et al.*, 1981b). Also, an enhanced excretion of organic matter will stimulate the growth of microorganisms around the root and, concomitantly, the use of oxygen. This eventually will lower oxygen levels. In this way, ferric chelation and reduction may go hand in hand. However, the capacity of iron-deficient roots to rapidly reduce ferric chelates is difficult to reconcile with the kinetics of ferric reduction by, e.g., caffeic acid, the most abundant "reductant" found in these roots (Römheld and Marschner, 1983b). Chlorogenic acid also has been proposed as a reductant (Hether *et al.*, 1984).

In 1982, new arguments were proposed for an enzymic reaction at the plasmalemma as the main cause of the reduction that could be measured directly at the root surface (Bienfait *et al.*, 1982b). This was supported by results from Marschner's group (Römheld and Marschner, 1983b; Barrett-Lennard *et al.*, 1983). The existence of a transmembrane electron transfer system in plant cells has already been shown by Craig and Crane (1981, 1982) for cultured carrot cells, and Federico and Giartosio (1983) have also interpreted the reduction of ferricyanide by intact maize roots on this basis.

As electron donors for the transmembrane reduction system with ferricyanide as an acceptor, both NADH (Craig and Crane, 1981, 1982; Federico and Giartosio, 1983) and NADPH (Sijmons *et al.*, 1984a) have been proposed. Craig and Crane (1981) were able to influence the reduction rate by addition of ethanol with or without pyrazole, an inhibitor of alcohol dehydrogenase. This led them to the conclusion that NADH was the electron donor in the cells they used. Sijmons and Bienfait (1983) reported that nitrotetrazolium blue was a highly effective inhibitor of the inducible system, and that the formation of its reduced precipitate in the cell could be prevented by extracellular Fe-EDTA. Recently, they showed that in roots of bean

plants the total amount and reduction state of NADP were strongly increased upon Fe deficiency. When ferricyanide was added, it could be shown that within 2 min the NADPH/NADP couple became more oxidized (Sijmons *et al.*, 1984a). Interestingly, the redox state of NADP in maize roots was not influenced by the iron status of the plant. The positive correlation between ferric chelate reduction capacity and NADPH level (Sijmons *et al.*, 1984b) suggests that the concentration by cytosolic NADPH, rather than the amount of electron-transferring systems in the plasma membrane, is rate-determining for the induced reduction capacity and is the relevant parameter under regulation by the iron status of the plant.

Reduction of extracellular ferric salts has a depolarizing effect on root cell membrane potential (Sijmons *et al.*, 1984c). This may explain the increased efflux of protons that accompanies ferricyanide reduction (Federico and Giartosio, 1983; Craig and Crane, 1981; Ze-sheng *et al.*, 1984). Yeast cells export about 1 H^+ per electron donated to ferricyanide (Crane *et al.*, 1982); with corn root cuttings a ratio of 0.31 H^+/e has been reported (Federico and Giartosio, 1983). In this case, export of K^+ might make up the difference.

Carrot cells were found to export 3–4 H^+/e; this high ratio was explained on the basis of a possible release of controls on another electron-transfer pathway, with oxygen as electron acceptor, or to a stimulating effect on the plasma membrane ATPase (Craig and Crane, 1981).

A system in corn roots capable of reducing extracellular NADH also could be obtained in soluble form (Lin, 1982a, b). The system in intact protoplasts exported 2 H^+ per NADH oxidized (Lin, 1984). The oxidation of extracellular NADH by intact roots or by the isolated system was not sensitive to DCCD (Lin, 1984), but in intact roots the increased efflux of protons, due to NADH oxidation, was completely inhibited. Since oxidation of NADH uses one H^+ per NAD^+ produced, DCCD should reduce H^+ export by 50%. This suggests that, upon oxidation of extracellular NADH, one proton is exported by the redox system itself, and one proton is driven out by a DCCD-sensitive proton pump.

Another mechanism which may play a role in the reduction of extracellular iron is the photochemical decay of ferric chelates (Frahn, 1958). Certain species grown in greenhouses under light poor in short wavelengths (low-pressure sodium lamps) developed leaves with very little chlorophyll, while the Fe content was normal. Fluorescent light rich in the short wavelengths remedied the typical Fe chlorosis (Brown *et al.*, 1979). The authors assumed that the mesophyll cells of these plants had a low capacity to reduce extracellular iron. The form in which iron was present around these cells was most probably ferric citrate, as this is the form in which it is transported in the xylem (Tiffin, 1966a, b, 1970). Brown *et al.* (1979) con-

cluded that the blue light relieved chlorosis by reducing the ferric citrate in the extracellular space, so that the resulting ferrous ions, less tightly bound by citrate and its decay products, could be taken up by the cells. This is the simplest explanation for the observed phenomenon, although an effect via a cellular photoreceptor (Jesaitis *et al.*, 1977; Leong and Briggs, 1982) cannot be ruled out.

Rhizodermal Transfer Cells

An interesting morphological response of dicotyledonous plants to iron deficiency is the formation of transfer cells in the epidermis of the roots (Kramer *et al.*, 1980; Landsberg, 1982; Kramer, 1983). These cells are characterized by a cell wall which on one side of the cell is thickened in a highly irregular way; this results in the formation of numerous wall invaginations, and a corresponding increase in the plasmalemma surface (Pate and Gunning, 1972). Transfer cells are found at places where high fluxes of transmembrane transport are occurring, e.g., between vascular elements and surrounding tissue. In the epidermis of Fe-deficient plants, it is the peripheral walls facing the root exterior which carry the labyrinth-like invaginations. According to Römheld and Kramer (1983a), the capacity of iron-deficient plants to form these transfer cells is correlated with the capacity of roots to lower pH. Thus, they are not found in the roots of grasses but in those of chlorotic sunflowers, beans, and tomatoes, all very active in lowering pH, which develop large numbers of transfer cells in the epidermis.

A function of transfer cells in extruding protons seems logical; e.g., this has also been proposed to be the case in the epidermal transfer cells of submerged water plants (Prins *et al.*, 1982). It is tempting to suppose that the convoluted plasma membrane surface also contains high concentrations of reducing enzyme systems, so that the high ferric reduction capacity of roots with transfer cells can be explained on this basis. However, there are two arguments against this possibility:

1. There is no correlation between the number of observable transfer cells and the reduction capacity of the roots (Römheld and Kramer, 1983a);
2. There is a close correlation between the amount of extractable NADPH and the capacity of the roots to reduce Fe-EDTA (Sijmons *et al.*, 1984b).

These arguments together suggest that the more important function of these transfer cells is to export protons. The tomato mutant T3238 fer, which is unable to develop ferric reduction capacity and pH lowering activity (Brown and Ambler, 1974a), is also unable to form transfer cells in the root when deprived of iron (Landsberg, 1981b).

Two Reduction Systems

A description of the ferric reduction system in plant roots in general is hampered by the fact that different laboratories work with different systems, cultured under different conditions. However, the data in the literature can be fitted together if one assumes that two transplasmamembrane electron transfer systems are operative: one constitutive and one controlled by the iron status of the plant. In Table I some characteristics of the two proposed electron transfer systems are summarized.

The constitutive or "Standard" system is thought to be present in all plant cells. It reduces ferricyanide but not ferric chelates such as Fe-EDTA, and its activity should not be influenced by the iron status of the plant. Its affinity for the electron donor, NADH or NADPH, is supposed to be high.

The inducible or "Turbo" system is only active in dicotyledonous plants and nongraminaceous monocots such as *Chlorophytum* (Brown, 1978; Römheld and Kramer, 1983a). It is able to reduce a wide variety of ferric chelates including ferricyanide, and its capacity is strongly increased during iron-deficient conditions. The proposed electron donor is NADPH (Sijmons *et al.*, 1984a), and it is the potential supply of electrons to this redox carrier that determines the capacity of the reductase. The affinity of NADPH is thought to be low. The system is active in the epidermal cells of the young roots (Ambler *et al.*, 1971; Brown and Ambler, 1974a; Marschner *et al.*, 1982).

The work of Craig and Crane (1981, 1982), Federico and Giartosio (1983), and Rubinstein *et al.*, (1983) would then relate to the constitutive system, as their cells and roots have been grown without nutritional limitations. This may also be the case for the system proposed by Novak for freshwater plants (Novak and Ivankina, 1978; Ivankina and Novak, 1980), and by Lin for maize roots (Lin, 1982a, b; 1984), where both systems use oxygen as the electron acceptor. The work on ferric chelate reduction (Olsen *et al.*, 1982; Römheld and Marschner, 1983b; Sijmons and Bienfait, 1983)

Table I. The Properties of Two Possible Transplasmamembrane Electron-Transfer Systems in Plant Cells

	Constitutive "Standard"	Inducible "Turbo"
Presence	All plants	Dicots and nongrass monocots
Localization	Ubiquitous	Epidermis of young lateral roots
Active with	Ferricyanide	Ferricyanide and Fe-EDTA
Natural electron acceptor	Oxygen	Ferric chelate
Electron donor	NADH or NADPH	NADPH
K_m for electron donor	Low	High
Function	Membrane polarization	Iron uptake

would relate to the system which is under the control of the iron status. The function of the inducible system in dicots and the nongraminaceous monocots is obvious: to increase the capacity of the root to maintain extracellular iron in the ferrous state in which it can be taken up.

Since grasses are thought to take up iron complexed with excreted siderophores, and since their response to iron deficiency is supposed to be an increase in the excretion of these chelators, the questions arise as to what is the function of the constitutive system, and what is its natural electron acceptor. The need for extracellular reducing equivalents to produce H_2O_2 via NADH for the synthesis of cell wall constituents has been documented, but malate, via malate dehydrogenase, is thought to reduce the extracellular NAD in the cell wall (Gross *et al.*, 1977). It is also possible that oxygen is the natural electron acceptor, and that the function of the redox chain in the plasmalemma is to export protons in order to build up a transmembrane potential, in a manner independent of the fusicoccin-stimulated ATPase (Poole, 1978; Marrè, 1979). In this respect, it is interesting to note that, in intact roots, fusicoccin only stimulated the proton extrusion in older root parts, far behind the zone of cell elongation (Römheld *et al.*, 1984). It goes beyond the scope of this review to compare the recent findings on transplasmamembrane electron transfer with theories on ion uptake (e.g., Conway, 1951; see also Jennings, 1976).

Regulation of the Ferric Chelate-Reducing System by the Iron Status of the Plant

Regulation of the ferric-reducing capacity is most probably not a matter of a "chlorosis" signal or a hormone transported from the chlorotic shoot to the roots. This was shown by Brown and co-workers by grafting experiments with Fe-efficient and Fe-inefficient soybean and tomato varieties (Brown *et al.*, 1958, 1971).

Olsen *et al.*, proposed that low-molecular-weight phenolics are excreted by the roots of Fe-deficient plants, possibly while lowering pH, and associated with a concomitant leakiness of the root cell membranes (Olsen *et al.*, 1982). They found increased activity of the enzyme *p*-coumarate hydroxylase which produces caffeic acid, and decreased activity of polyphenol oxidase which converts it to other products, all in comparison with the mutant T 3238 fer (Olsen *et al.*, 1981a). However, as discussed before, strong arguments have been raised against excreted compounds producing the high reduction capacity of the root surface.

Pound and Welkie (1958) and Welkie and Miller (1960) noted an accumulation of flavins in the medium surrounding the roots of Fe-deficient

tobacco plants. This phenomenon also has been reported for sunflower (Venkat Raju *et al.*, 1972) and sugar beet (Nagaraja and Ulrich, 1966), but it is not a general phenomenon with dicots. It is possible that flavins take the place of Fe–S centers in the cell when iron is getting short, as has been shown for flavodoxin in algae (Knight *et al.*, 1966; Zumft and Spiller, 1971). This in itself does not explain the increased capacity of the transmembrane electron-transfer system, unless it can be shown that a flavin controls its rate. According to Landsberg, indoleacetic acid accumulates in the roots of Fe-deficient plants. Such an accumulation was proposed to cause the pH decrease and several morphological changes in the roots, including the formation of transfer cells, in which plastids could be involved in the production of reductants (Landsberg, 1981a, 1982). The increased level of IAA in the roots would be the result of increased production and transport from the shoot (see, however, Brown *et al.*, 1958, 1971), or a decreased capacity of the roots to inactivate IAA (Römheld, 1979). Sijmons *et al.*, (1984a) proposed that the increased level of NADPH in Fe-deficient roots may be caused by the inhibition of synthetic pathways in which iron-containing enzymes are involved, and which need large amounts of NADPH.

The colorful variety of proposed schemes illustrates how little is known of the Fe-deficiency reactions in plants at the cellular level. If Fe deficiency induces the activity of a transmembrane electron-transfer system, is the induction merely a result of NADPH accumulation or is protein synthesis also involved? How does one gene in tomatoes control the development of rhizodermal transfer cells in response to Fe deficiency, as well as the activation of ferric reduction capacity? What is the difference between the two groups of plants that causes the grasses, on the one hand, to excrete siderophores, and the other monocots *plus* the dicots, on the other hand, to activate their ferric reduction capacity and to make rhizodermal transfer cells?

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