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Review–Hypothesis

Siderophores, the iron nutrition of plants, and nitrate reductase

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1. INTRODUCTION

Iron is ubiquitous in terrestrial environments [1]. It is, however, very sparingly soluble under aerobic conditions as ferric hydroxides are formed and the solubility product approaches 10^{-38} [2]. Microorganisms have solved the problem of iron acquisition under aerobiosis by synthesizing siderophores, i.e. high-affinity Fe^{3+}-chelating compounds with formation constants ranging from approx. 10^{20} to 10^{50} [3]. Siderophores are organic compounds comprised of either hydroxamates or catecholates (fig.1) [1,3,4]. A new category of siderophores, termed the 'miscellaneous' class, has been recently recognized [5] and this group differs in chemical structure from the previous classes of siderophores, most likely containing imino or amino carboxylic acids. The reader is referred to reviews [1–7] for a more detailed discussion of siderophore chemistry and microbial iron nutrition.

The competition for Fe^{3+} results in the excretion of siderophores from microbes into the environment. Powell et al. [8] noted minimal siderophore concentrations of 3 nM desferrioxamine methanesulfonate equivalents (the hydroxamate siderophore used as a standard) in each of 57 soils examined. Siderophores were thus noted in grassland, coniferous and deciduous forest, and mixed herbaceous vegetation soils from across the United States. In 19 soils, concentrations of siderophores varied from 2.7 to 34 nM desferrioxamine methanesulfonate equivalents with a mean concentration of 12 nM. A 1:1 (soil-water) extract of a sandy clay loam [9] contained 78 nM siderophores. Similar results were noted from soils in Texas [10] and the hydroxamate siderophore schizokinen [11] was recovered and identified from a rice field soil. Hydroxamate siderophores are not only present in but are also capable of chelating and mobilizing iron from soil as desferrioxamine B, desferrichrome [12] and (deferri-)rhodotorulic acid [13] removed iron from acid and alkaline soils [12] as well as silicate rocks and Mt. St. Helens' ash [13]. An interesting question raised by these studies is that of iron acquisition by plants; how do plants acquire the iron they require when the soils in which they are growing contain numerous microbes and microbial siderophores?

2. ASSIMILATION OF FERRISIDEROPHORE IRON BY PLANTS

Kloepper et al. [14,15] reported that plant growth-promoting rhizobacteria (PGPR) enhanced the growth of potatoes, sugar beets and radishes, noting yields of up to 144% of controls. Among the PGPR were members of the genus *Pseudomonas*. The siderophore synthesized by one of the pseudomonads was later purified, given the name pseudobactin and the structure elucidated [16]. Competition for Fe^{3+} between the PGPR and plant deleterious microbes is thought to result in

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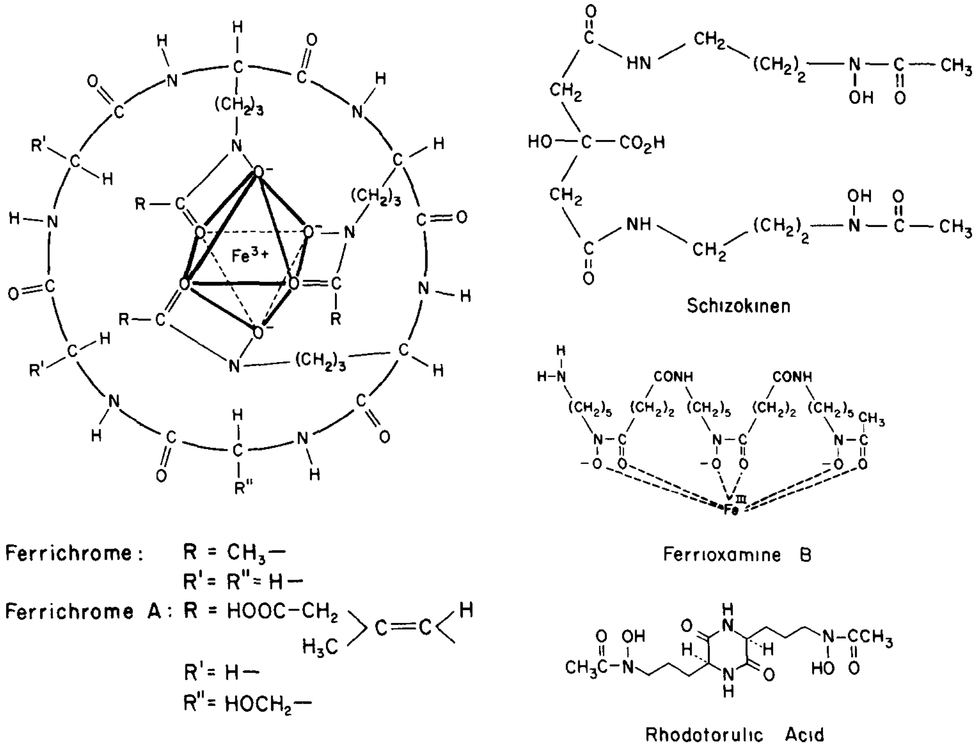

Fig.1. Structures of the hydroxamate siderophores ferrichrome, ferrichrome A, ferrioxamine B, rhodotorulic acid and schizokinen.

the protection of the plant by the PGPR [14,17] and has initiated the examination of PGPR and similar microbes as biological control agents [17,18]. The situation may be more complex, however, as antibiotic production by PGPR and similar microbes may also play a role in plant pathogen suppression. For example, a recent study presented evidence that the ferrisiderophore, not the deferrisiderophore, was in part responsible for inhibition of the agent of black root rot, i.e. *Thielaviopsis basicola* [47]. The pseudomonad which synthesized the siderophore also made cyanic acid and antibiotics and these agents were similarly effective in limiting the growth of the pathogen.

That plants can assimilate the iron of ferrisiderophores was shown when ^{55}Fe in ferrichrome was assimilated into the shoots of oats [19]. Ferrioxamine B can supply iron to sunflowers [20] and iron from ferrichrome and ferrichrome A was assimilated by duckweed and tomato plants [21]. Tomatoes are also able to assimilate the iron of [^{59}Fe]ferrioxamine B [22]. Tomato is adept at using the iron of a number of siderophores as it also utilizes ferrirhodotorulic acid [23]. This ferrisiderophore relieves the iron-deficiency stress imposed by growth in an iron-poor environment. Furthermore, the iron-efficient T3238 FER strain of tomatoes was much more adept at using ferrirhodotorulic acid as an iron source than was the counterpart iron-inefficient strain T3238 fer [23]. A recent study similarly concluded that iron efficiency in tomatoes may be critically linked to bacterial products, such as siderophores, as iron-efficient tomatoes were efficient only when bacteria were present in the rhizosphere [46].

Agrobactin, a catechol siderophore, functions like the hydroxamate siderophores with respect to supplying iron to bean and pea plants [24]. Autoradiograms clearly showed that the iron of [^{59}Fe]ferriagrobactin was translocated into the shoot of the bean plant and then disseminated throughout the plant. The iron so delivered to the plant was available for physiological incorporation into plant tissues as evidenced by the stimulation of chlorophyll *a* and *b* synthesis in the agrobactin-treated plants [24].

Assimilation of the C of siderophores by the plant remains an unresolved point although [^{14}C]ferrichrome is taken up by clover [25] and hydroxamate siderophores were detected in the leaves of several crop plants [26]. Not all siderophores, however, are capable of supplying iron to plants as Fe^{3+} assimilation was inhibited, resulting in a decreased synthesis of chlorophyll in maize and pea plants when pseudobactin was added to the nutrient solution [27].

3. PLANT IRON ASSIMILATION MODELS

Many plants respond to a lack of available iron by excreting reductants, such as caffeic acid [28], and by lowering the rhizosphere pH [28–30]. Earlier studies hypothesized that the lowered pH aided in inorganic iron solubilization and that the reductants functioned to reduce Fe^{3+} to Fe^{2+} before being assimilated by the plant. The demonstration of Fe^{3+} reduction at the root surface [29,31] resulted in the proposal that a 'ferric ion reductase' is an intimate component of iron assimilation in plants.

Romheld and Marschner [32] noted that iron-deficient peanut plant roots had an optimal pH of about 5.0 for the reduction of Fe^{3+}. Furthermore, the observed rate of Fe^{3+} reduction by phenolic reductants (*p*-coumaric, chlorogenic and caffeic acids) had a pH optimum of greater than 8.0 and was some 200-fold too slow to account for the iron acquisition of the plant. These authors suggested that an "enzymic reduction of ferric ion on the plasmalemma of cortical cells of roots" was a key facet of iron assimilation by iron-deficient peanut plants [32]. Bienfait and colleagues [31,33], studying bean plants, reached similar conclusions concerning the involvement of an Fe^{3+}-reducing enzyme in the cortex or epidermis cell plasmalemmas. In addition, data indicating the use of cytosolic NADPH as the electron source for Fe^{3+} reduction were also presented [33].

Romheld and Marschner [34] noted that while a number of monocotyledonous and most dicotyledonous plants invoke the NADPH-requiring reductase, the monocotyledonous grasses lack this response. The grasses, exemplified by barley [35], however, can solubilize iron from low-solubility inorganic iron compounds. The grasses apparently sequester the iron needed for growth by secreting their own Fe^{3+} chelators termed phytosiderophores. These chelators are composed of nonproteinogenic amino acids and have been isolated from barley (mugineic acid), beer barley (distichonic acid), oats (avenic acid A), wheat (2′-deoxymugineic acid), rye (3-hydroxymugineic acid), and tobacco (nicotianamine) [36]. Barley assimilates iron from ferriphytosiderophores 100–1000-times faster than from other ferri-chelators, including ferrisiderophores. In addition, reduction of the ferriphytosiderophores by barley does not occur during iron assimilation, as occurs with non-graminaceous monocotyledonous and dicotyledonous plants [34].

Romheld and Marschner [34,37] thus proposed that two distinct strategies are employed by most nongraminaceous species (strategy I) and by the graminaceous species (strategy II). Strategy I invokes the release of protons and reductants coupled with a plasma membrane-bound Fe^{3+} reductase. Strategy II is dependent upon the release of phytosiderophores from the graminaceous plants and the subsequent ferriphytosiderophore assimilation, as an intact unit, by the plant. The proposed 'strategies' give rise to physiological and biochemical questions. Whether the phytosiderophores are transported and made to yield their Fe^{3+} in a manner analogous to those noted in microbes [1,4,6,7], i.e. by reduction of the ferrisiderophore by a cellular ferrisiderophore reductase, is one such question. Another is the isolation and identification of the inducible Fe^{3+} reductase of the dicotyledonous and nongraminaceous monocotyledonous plants.

4. PLANT NITRATE REDUCTASE AS A SIDEROPHORE REDUCTASE

In higher plants, the first enzyme of the nitrate assimilatory pathway is nitrate reductase [38]. Most higher plant nitrate reductases are specific for NADH [38], although bispecific NAD(P)H nitrate reductases have been demonstrated in many tissues including soybean leaves and cotyledons [38], and corn roots and scutella [38]. Although the physiological activity of this enzyme is generally regarded as the pyridine nucleotide reduction of nitrate to nitrite, alternative electron acceptors have been identified [39]. These include mammalian cytochrome *c*, dichlorophenolindophenol, ferricyanide, and menadione [39]. Recently, it was established that nitrate reductase could catalyze the reduction of an iron-citrate complex [40]. Based on these observations, and on the fact that a partially purified siderophore reductase activity from soybean seedlings had characteristics of a nitrate reductase and would probably copurify with nitrate reductase [41], the capacity of squash cotyledon nitrate reductase to function as a siderophore reductase was subsequently examined [42,43].

The following characteristics of this activity were observed. A number of ferrisiderophores were reduced by nitrate reductase [43]. Included were ferrichrome, ferrichrome A, ferrirhodotorulic acid, ferrischizokinen, ferrioxamine B, and an unnamed catecholate siderophore isolated from *Aeromonas hydrophila* [43]. Catalytic activity was maximal at either pH 4 or 5, which is different from the pH of maximal activity with nitrate, i.e. 7.5. Rates of reduction varied from 2 to 17% of the rate of nitrate reduction, and apparent Michaelis constants were in the micromolar range [43]. Since antibodies against nitrate reductase inhibited the ferrisiderophore reductase activity, it was concluded that this activity was catalyzed by nitrate reductase. Furthermore, nitrate reductases from various fungi and bacteria have also been shown to catalyze Fe^{3+} reduction [44,45].

Although it cannot be conclusively stated that nitrate reductase functions as the only siderophore reductase in higher plants, pH regulation of the nitrate and siderophore reductase activities is plausible. As plant roots are known to decrease the pH of their surrounding environments to values which would favor ferrisiderophore reduction over nitrate reduction [28–30], a possible mecahnism for the regulation of the activity of this biocatalyst exists. Fig.2 depicts an electron transport scheme for nitrate reductase functioning as a ferrisiderophore reductase.

Based on our observations with squash cotyledon nitrate reductase, we hypothesize that higher plant nitrate reductases are involved in iron acquisition of plants from siderophores. One may speculate that nitrate reductases are involved in the reduction of phytosiderophores such that iron is released in graminaceous plants. Further experimentation is needed to determine if ferrisiderophores are both assimilated into plant cells and made to release the iron they contain by reduction due to nitrate reductase or other ferrisiderophore reductases. Similarly, experiments to determine if phytosiderophores are reduced by the various nitrate reductases of both the graminaceous and nongraminaceous plants need to be performed. A clearer understanding of the role of nitrate reductases in the iron nutrition of plants would thus be achieved.

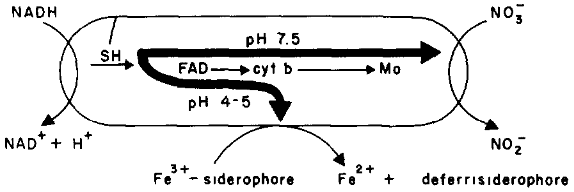

Fig.2. Electron transport scheme for the plant enzyme NADH: nitrate reductase during the reduction of nitrate and ferrisiderophores. The thinner lines represent the pathways of electron transport while the bold-face lines represent the pH control of electron transport.

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