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Utilization of Microbial Siderophores in Iron Acquisition by Oat[1]

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ABSTRACT

Iron uptake by oat (*Avena sativa* cv Victory) was examined under hydroponic chemical conditions that required direct utilization of microbial siderophores for iron transport. Measurements of iron uptake rates by excised roots from the hydroxamate siderophores, ferrichrome, ferrichrome A, coprogen, ferrioxamine B (FOB), and rhodotorulic acid (RA) showed all five of the siderophores supplied iron, but that FOB and RA were preferentially utilized. FOB-mediated iron uptake increased fourfold when roots were preconditioned to iron stress and involved an active, iron-stress induced transport system that was inhibited by 5 millimolar sodium azide or 0.5 millimolar dinitrophenol. Kinetic studies indicated partial saturation with an apparent K_m of 5 micromolar when FOB was supplied at 0.1 to 50 micromolar concentrations. Whole plant experiments confirmed that 5 micromolar FOB was sufficient for plant growth. Siderophore-mediated iron transport was inhibited by Cr-ferrichrome, an analog of ferrated siderophore. Our results confirm the existence of a microbial siderophore iron transport system in oat which functions within the physiological concentrations produced and used by soil microorganisms.

Siderophores are microbially produced, iron-chelating agents that can increase and regulate the availability of iron in the plant rhizosphere (9, 15). Recently, these compounds have come under investigation in plant nutrition because siderophores have also been found to serve as iron sources for plants (6, 19). Over 80 different siderophores of bacteria and fungi have been isolated (17). Among these, plants use the hydroxamate siderophores, FC,[2] RA, and FOB; the catechol siderophore, agrobactin; and the mixed ligand, catechol-hydroxamate-hydroxy-acid siderophores produced by *Pseudomonas* (8, 15). However, not all siderophores may be used by plants (1), and individual plant species and varieties have different abilities to utilize specific siderophore types (5, 11). Such differences suggest there are at least two mechanisms for siderophore-mediated iron transport.

To acquire iron from siderophores, microorganisms employ receptor protein complexes that bind and transport the intact iron chelate into the cell or remove iron from siderophores at the cell surface (14). Though less understood, plants might also have siderophore-iron transport systems or, under reduced soil conditions, might acquire inorganic iron buffered by dissociation of siderophores in the plant rhizosphere (5, 6). Prior research on plant iron nutrition has examined synthetic chelates that donate iron to plant roots by extracellular dissociation (10). Dissociation occurs when plants alter the redox and pH conditions of the rhizosphere to increase solubility of inorganic iron. Also, iron-efficient dicotyledonous plants may remove ferric iron from synthetic chelates with a cell surface reductase that releases inorganic ferrous iron into the root apoplast (2). In contrast, plants appear to utilize microbial siderophores differently since siderophores do not release iron into the apoplast of dicotyledonous plants nor readily dissociate above pH 4. In studies postulating siderophore transport, Smarrelli and Castignetti (22) have shown that inorganic iron may be released from hydroxamate siderophores by plant cytosolic NADH: nitrate reductase. Alternatively, Romheld and Marschner (20) have suggested a model that involves a plasmalemma chelate binding site with a reductase-carrier protein for inorganic iron.

Although current methods do not allow the precise determination of individual siderophore concentrations, bioassays of soil-water extracts indicate that hydroxamate siderophores are a predominant siderophore-type in soils (3, 16) and that they occur in elevated concentrations in the rhizosphere (18). In addition to the milieu of microbial siderophores, plants also produce organic acids, such as citrate and malate, which may solubilize iron at low pH (4), and 'phytosiderophores' that may function in iron uptake by monocotyledonous grasses (21, 23, 25). However, in soils, microbial siderophores have much higher affinity for iron than plant-produced organic acids (4) or phytosiderophores (6). This suggests that plants, like microorganisms, have multiple systems for iron transport to use the various chelates that sequester iron. For example, the enteric bacterium *Escherichia coli* produces and transports the catechol-siderophore, enterobactin, but also has at least four other separate receptor/transport systems for iron citrate, FC, FOB, and other hydroxamate siderophores produced by soil bacteria and fungi (6, 14).

In this study, we investigated the existence of a putative siderophore-mediated iron transport system in a monocot grass species, oat, in experiments that examined iron acquisition from five representative hydroxamate siderophores. To examine direct use of siderophores, the experiments were conducted under defined chemical conditions that precluded extracellular dissociation of siderophore-iron or plant use of phytosiderophores. Iron uptake rates were measured within the physiological concentration range produced by soil microorganisms to characterize iron uptake kinetics and to determine possible specificity for different siderophore types. Siderophore-mediated iron transport was further examined in experiments with siderophore chelated with chromium. This Cr-siderophore served as an Fe-siderophore analog; in microbial iron transport this analog has been shown to competitively inhibit utilization of ferrated siderophore (7, 17). Our results confirmed the existence of a siderophore-mediated iron transport system in oat and suggested that siderophores produced by rhizosphere microoorganisms can supply iron to plants that have mechanisms for utilizing these compounds under iron-limiting conditions.

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[2] Abbreviations: FC, ferrichrome; RA, rhodotorulic acid; FOB, ferrioxamine B; FCA, ferrichrome A; COP, coprogen; CrFC, Cr-ferrichrome.

MATERIALS AND METHODS

Siderophores. FC was produced in batch culture of *Ustilago sphaerogena* Burrill (ATCC 12421) using the medium of Wiebe and Winklemann (26). Inoculum was started in 100-ml subcultures and added at 10 ml/1000 ml of the same medium for batch production cultures. Production cultures were incubated at 30°C for 10 to 12 d with shaking at 250 rpm. The culture was then centrifuged at 6000*g* for 15 min, after which the supernatant was amended with 0.02% (w/v) $FeCl_3$ and concentrated by rotary evaporation *in vacuo* at 50°C. Extraneous protein was precipitated by extraction with methanol and removed by filtration through Whatman No. 1 paper. Methanol was removed by evaporation, and the resulting clear red solution was applied to an Amberlite column (XAD-2, Sigma) of 2.5 × 40.0 cm (d × h). After washing with three bed volumes of deionized water, FC and FCA were eluted with acetone-water (1:1) and separated on DEAE-cellulose eluted with 0.1 M phosphate buffer at pH 7. Purity was determined by scanning absorption spectroscopy and by silica thin-layer chromatography. FOB was obtained as Desferal (desferrioxamine B mesylate) from Ciba Geigy. RA was obtained from Porphyrin Products, Logan, UT. Coprogen was obtained as a gift from Dr. Carl Carrano. Concentrations of all siderophores were determined by absorbance using published extinction coefficients (17). Siderophores were deferrated by the hydroxyquinoline procedure described by Wiebe and Winklemann (26). Unless described otherwise, all siderophores were prepared with 10% excess desferrisiderophore.

Preparation of Radiolabeled and Chromium-Siderophore. Radiolabeled ^{55}Fe-siderophores were prepared by chelation of deferrated siderophore with inorganic $^{55}FeCl_3$ (New England Nuclear, specific activity: 21.3 mCi/mg $^{55}FeCl_3$). Each siderophore was prepared as a 10-ml stock solution containing 100 μCi ^{55}Fe with desferrisiderophore fourfold in excess of total iron. Although the chelation reaction is visibly instantaneous at high concentrations, the chelates were prepared 24 h in advance of the experiments to allow complete reaction with iron at very low concentration. Individual treatments in experiments utilized 10 μCi of labeled chelate with the remainder of the desired concentration supplied as nonradiolabeled ferrated siderophore. CrFC was prepared by addition of fourfold excess chromium (III) as $CrK(SO_4)_2 \cdot 12\ H_2O$ to 1 mM desferriferrichrome. The mixture was allowed to stand overnight at 60°C, after which nonchelated inorganic chromium was removed by separation on a column of Chelex 100. Alternatively, CrFC was purified on Amberlite XAD-2. Complete chelation with chromium was determined by the inability of the slightly green-colored CrFC to react with iron. Final concentration of CrFC was determined by inductively coupled plasma analysis for chromium (Colorado State University Soils Testing Laboratory), and purity was determined by silica gel thin-layer chromatography in MeOH:H_2O (80:20) (7).

Calculation of Direct-Use Experimental Conditions. To examine direct siderophore-mediated iron transport by plant roots, it was necessary to maintain conditions that inhibited extracellular dissociation of inorganic iron in equilibrium with ferrated siderophores. Siderophores have next greatest affinity for protons which may displace iron. Levels of inorganic iron in equilibrium with chelated iron were calculated from the general equilibrium equation for siderophores with iron and protons (6, 17):

$$\log \text{M}\ [Fe^{3+}] = (-PC) - 3\ pH - \log [LH_3]/[FeL]$$

where L = ligand and PC (protonation constant) = 3.4 and 2.13 for FOB and FC, respectively (17). As shown by this equation, levels of inorganic iron maintained by siderophore dissociation are pH dependent, such that soluble Fe^{3+} decreases 1000-fold for every unit increase in pH. At pH 7 in equilibrium with FOB and 10% desferri-FOB, the concentration of $Fe^{3+} = 10^{-23.4}$ M.

To determine the concentrations of reduced ferrous iron, the above equation was solved for equilibrium with ferrous iron where $Fe^{3+} + e^- \leftrightharpoons Fe^{2+}$, $\log K = 13.04$ (10). When solved for equilibrium with the representative siderophore, FOB:

$$\log \text{M}\ [Fe^{2+}] = 9.64 - 2\ pH - (pe + pH) - \log [LH_3]/[FeL]$$

As shown by this equation, Fe^{2+} decreases 100-fold for every unit increase in pH and 10-fold for every unit increase in pe + pH, where pe = Eh/59.2. In solution at pH 7, pe + pH = 18, and 10% excess desferri-FOB, $Fe^{2+} = 10^{-21.4}$ M. Under these defined conditions that were maintained in siderophore iron uptake studies, soluble inorganic iron was presumed to be unavailable for plant uptake.

Plant Culture. Oat seeds (*Avena sativa* cv Victory) were planted in silica sand. Three days after germination, the seedlings were removed from the sand, rinsed in deionized water, and established in aerated hydroponic culture. The nutrient solution was a modified, iron-free Hoagland solution containing: 3 mM KNO_3, 2 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4 \cdot H_2O$, 25 μM KCl, 12 μM H_3BO_3, 1 μM $MnSO_4 \cdot H_2O$, 1 μM $ZnSO_4 \cdot 7H_2O$, 0.25 μM $CuSO_4 \cdot 5H_2O$, and 0.25 μM H_2MoO_4. Solution pH was buffered at pH 7.2 to 7.4 by solid phase $CaCO_3$ (0.1 g L^{-1}). Redox of the aerated nutrient solutions as measured against a platinum electrode was maintained at greater than pe + pH = 18. The plants were grown under incandescent and VHO white fluorescent lamps (500 μmol m^{-2} s^{-1}) in a growth chamber (16 h day:8 h night cycle) at 25°C day; 20°C night temperatures.

Whole Plant Siderophore-Iron Uptake Experiments. Whole plant uptake studies were conducted with plants produced in 500-ml containers with six plants per container and three or four replicate containers per treatment. In experiments examining plant utilization of FOB without radioactive iron, the nutrient solutions were amended with 0, 0.1, 1, 5, 10, and 50 μM FOB with desferri-FOB 20% in excess of total iron. Plants were grown for 21 d, during which time the nutrient solutions were replaced every 2 d. At the end of the growth period, the plants were collected for analysis of plant dry weight, leaf Chl content, and foliar iron. Foliar iron concentrations were measured in acid digests of plant leaf material by inductively coupled plasma analysis at the University of Florida Soil Testing Laboratory. In whole plant experiments examining the effect of CrFC on iron acquisition, plants were grown for 14 d in nutrient solutions without iron. The visibly iron-chlorotic plants were then transferred to nutrient solutions containing 0.02 μM radiolabeled ^{55}Fe-ferrichrome with and without 10 μM CrFC and were grown for an additional 6 d after which the plant shoots were harvested for analysis of ^{55}Fe-content by liquid scintillation counting.

Siderophore-Iron Transport by Excised Roots. Iron-stressed and iron-sufficient plants were grown in 20-L containers with 40 plants per container in aerated hydroponic culture as above. To obtain large quantities of root material, iron-stressed and iron-sufficient plants were initially grown for 10 d in nutrient solutions with iron supplied as 50 μM Fe-EDTA. After 10 d, the nutrient solutions were changed and the plants were grown an additional 10 to 14 d with and without Fe-EDTA, at which time plants grown without iron demonstrated visible symptoms of iron chlorosis. New root growth from iron-stressed and iron-sufficient plants was excised, rinsed three times in nutrient solution without iron, cut into 1.5 cm segments, and placed in perforated disc-shaped plastic capsules (VWR Scientific) weighted with glass beads. Experiments were conducted in 100-ml glass jars with 80 ml of radiolabeled siderophore in nutrient solution without $CaCO_3$ but adjusted to pH 7 by addition of 0.1 M NaOH. After the roots were immersed in the treatment solutions, the containers were loosely capped to prevent escape of radioactive aerosols, and the solutions were aerated to maintain oxidized conditions and solution mixing. Initial experiments demonstrated that uptake was linear over 6 h. Subsequent uptake

experiments were limited to 3 to 4 h. At the end of the uptake period, the roots were removed from radiolabeled solutions, rinsed twice with ice-cold, iron-free nutrient solution, and allowed to desorb in 500 ml of nutrient solution at 3°C for 1 h with solution changes at 10 and 30 min. The roots were oven-dried at 60°C for 24 h, weighed (20 mg per replicate sample, average dry weight), and acid digested in 600 μl of H_2SO_4 and 400 μl of H_2O_2. A 100 μl sample of the clear digest mixture was sequentially mixed with 1 ml of water and 10 ml of Scintiverse (Fischer Scientific). ^{55}Fe content was determined by liquid scintillation counting with counts corrected to dpm/mg by external standard ratio. Uptake data were corrected for nonspecific adsorption of chelated iron by determination of radioactive iron in root samples incubated for 2 min in radiolabeled nutrient solutions and processed as above. Iron uptake was expressed as μg Fe g^{-1} root dry weight h^{-1}. Since the rates of iron uptake from RA which forms a 3:2 complex with iron are not directly comparable with the other siderophores having a 1:1 stoichiometry, uptake rates from RA_3Fe_2 were expressed on an equivalent basis of total soluble iron, *e.g.* 3 μmol of RA formed 1 μmol of RA_3Fe_2 that supplied 2 μmol of Fe.

Statistical Methods. Data were analyzed by analysis of variance. When ANOVA generated a significant F value ($P = 0.05$), treatment means were compared by Tukey's HSD (honestly significant difference). All experiments were repeated at least twice.

RESULTS

Direct Use of Microbial Siderophores. Under conditions that required siderophore-mediated iron transport, iron-stressed oat roots acquired iron from all five of the hydroxamate siderophores that were tested (Fig. 1). Among the five siderophores, oat demonstrated significant specificity ($P = 0.01$) for iron acquisition from RA and FOB, which supplied seven- to eight-fold greater iron in comparison to FC, FCA, COP. In experiments comparing all five siderophores with the same plant material at 1 μM iron concentration, the mean uptake rate from FC, COP, and FCA was 0.18 μg Fe g^{-1} root dry weight h^{-1}, whereas RA and FOB supplied 1.25 and 1.59 μg Fe g^{-1} root dry weight h^{-1}, respectively.

Uptake rates varied by approximately 20% in replicated experiments with similarly grown plant material and solution preparations, but the rates were consistent in showing that FOB and RA were better utilized than FC, FCA, and COP at 1, 5, and 10 μM concentrations. At 10 μM concentration, FOB and RA supplied approximately 6.25 μg Fe g^{-1} dry weight h^{-1}, whereas FC and FCA and COP supplied approximately 1.6 and 0.8 μg Fe g^{-1} dry weight h^{-1}, respectively (Fig. 1). Relative differences among the siderophores diminished at the 0.1 μM concentration.

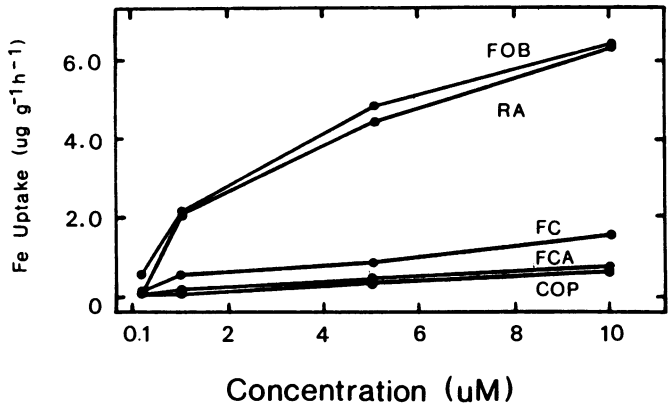

FIG. 1. Rates of iron uptake from the hydroxamate siderophores, FOB, RA, FC, FCA, COP by excised roots in nutrient solutions containing 0.1, 1.0, 5.0, and 10 μM siderophore.

Iron uptake rates increased in direct proportion to the concentration of FOB and RA when supplied at 0.1 to 1 μM. However, the slope of the uptake curve *versus* siderophore concentration (Fig. 1) showed that uptake rates were partially saturated with only a 3- to 4-fold increase over the next 10-fold increase in concentration from 1 to 10 μM siderophore. Further experiments confirmed partial saturation kinetics with FOB supplied over a 0.1 to 50 μM concentration (50 μM FOB: 11.2 μg g^{-1} h^{-1}, data not plotted). With partially saturable iron uptake kinetics over relatively few concentration points, the precise K_m and V_{max} parameters were not obtainable by Lineweaver-Burk plot analysis. However, the breakpoint at which saturation began to occur, as well as calculation of the half maximum uptake rate with 50 μM FOB, indicated an apparent K_m of 5 μM for FOB utilization by iron-stressed roots.

Effect of Iron Stress on Siderophore-Iron Transport. Precondition of oat seedlings to iron stress significantly increased the iron uptake rate by excised roots supplied with 5 μM FOB. After 1, 2, 3, and 6 h in radiolabeled nutrient solution, iron-stressed roots acquired four-fold greater quantities of iron than roots from iron-sufficient plants (Fig. 2). Experiments with metabolic inhibitors showed that iron uptake by iron-stressed plant roots involved an active transport system that was induced by precondition to iron stresss. Iron transport by iron-stressed roots was inhibited in the presence of 5 mM sodium azide or 0.5 mM dinitrophenol (Table I), whereas iron transport by roots of iron-sufficient plants was not inhibited and was not significantly different from that of iron-stressed roots in the presence of inhibitors. This suggests that uptake by iron-stressed roots involved the summed contribution of passive and active uptake components.

Utilization of FOB by Whole Plants. The concentration of FOB required to maintain plant growth in hydroponic culture

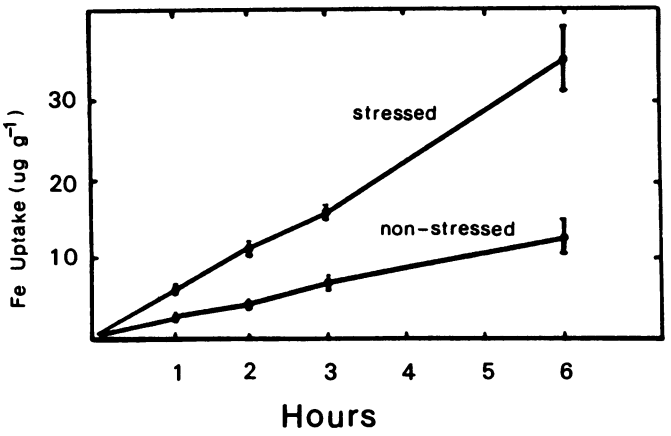

FIG. 2. Effect of preconditioning to iron stress on iron uptake from 5 μM FOB by excised roots of oat after 1, 2, 3, and 6 h. Uptake by iron-stressed roots and iron-sufficient roots was significantly different at all time points ($P = 0.05$) by Tukey's HSD. Bar = 2 SE.

Table I. *Effect of 5 mM Sodium Azide or 0.5 mM Dinitrophenol on Iron Acquisition (10^3 dpm mg^{-1} dry weight) from 5 μM FOB by Iron-Stressed and Iron-Sufficient Excised Oat Roots*

Different letters indicate significant differences between treatments ($P = 0.05$) By Tukey's HSD.

Treatment	FOB	+ Sodium Azide	+ Dinitrophenol
Fe-stressed	25.6 a	12.4 b	11.4 b
Fe-sufficient	7.0 b	11.6 b	12.6 b

was examined in whole plant experiments with nonradioactive FOB. Preliminary experiments indicated that FOB may be subject to microbial degradation in long-term nutrient solution culture of plants. The nutrient solutions were changed every 2 d to prevent depletion of siderophore-iron by plant uptake at low concentrations, to maintain fixed concentrations (periodically assayed by colorimetry), and to avoid the possible accumulation of phytosiderophores. After plant growth for 21 d in nutrient solutions containing 0, 0.1, 1, 5, 10, and 50 μM FOB (with 20% excess desferri-FOB), analysis of plant dry weight and Chl content showed the peak significant (P = 0.05) growth response to siderophore-iron was obtained at 5 μM concentration (Fig. 3). After 21 d, plants grown without FOB had stopped growing and were extremely iron chlorotic with necrotic leaf tissue. At this stage, iron deficiency was irreversible as similarly grown plants showed no recovery after addition of iron. Plants supplied with 0.1 and 1.0 μM siderophore were significantly larger and less chlorotic than plants grown without iron but were iron-deficient. At siderophore concentrations of 5, 10, and 50 μM FOB, the iron deficiency was alleviated, and there were no significant differences in either plant biomass or shoot Chl content.

As was observed with excised root experiments, shoot iron content data showed that iron uptake by whole plants was partially saturated at 10 μM FOB. Leaf tissue from whole plants grown with 0, 1, 5, 10, and 50 μM FOB contained 32, 36, 56, 88, 126, and 146 μg iron g^{-1} dry weight, respectively. Calculations based on total plant dry weight and shoot iron content showed that iron uptake rates with excised roots were not commensurate with shoot iron contents determined in long-term studies with whole plants.

Inhibition of Iron Uptake by Cr-Siderophore. Experiments were conducted with whole plants and excised roots to determine possible competitive inhibition of iron uptake from siderophores or from inorganic iron by CrFC, a purported inert analog of ferrated siderophore (7, 17).

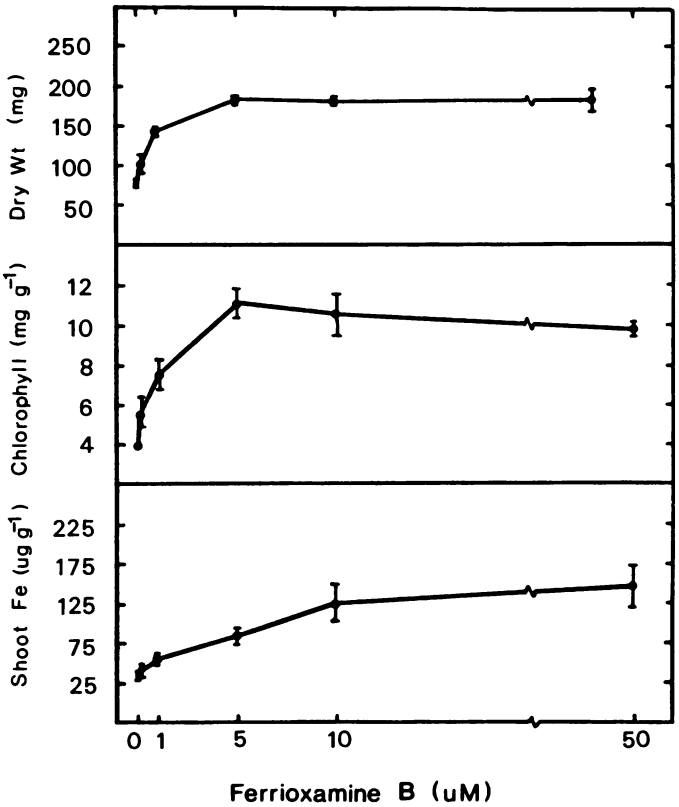

FIG. 3. Effect of siderophore concentration on plant dry weight (top), Chl content (middle), and shoot iron content (bottom) of hydroponically grown oat seedlings grown for 21 d with iron provided as FOB over a range of concentrations. Bar = 2 SE.

In whole plant experiments with solutions containing either 0.02 μM [^{55}Fe]FC or 0.02 μM initially soluble $^{55}FeCl_3$, addition of 10 μM CrFC significantly inhibited (P = 0.01) iron uptake by whole plants grown for 6 d in hydroponic culture (Table II). Shoot content of radiolabeled iron was reduced 8-fold in plants supplied with FC and 21-fold in plants grown with $FeCl_3$. To measure any iron solubilized by phytosiderophores in treatments with inorganic iron, total iron in solution was monitored in 1 ml aliquots of nutrient solution sampled at 24 h intervals over the 6-d uptake period. In solutions with FC, soluble iron decreased slowly over the 6-d period and was not affected by addition of CrFC. In treatments with inorganic iron, soluble and colloidal iron decreased rapidly over the first 24 to 48 h to levels approaching equilibrium with inorganic ferrosic hydroxide (10). After precipitation, soluble inorganic iron levels in solutions with $FeCl_3$, with or without CrFC, remained at less than 1 nM concentration, the detection limit by scintillation counting at the specific activity of radioactive iron used in these experiments.

Control experiments indicated that the reduction in iron content of plants grown with CrFC was not a result of chromium toxicity. Addition of 10 μM inorganic chromium as soluble $CrK(SO_4)_2$ had no effect on iron uptake by whole plants from FC or from $FeCl_3$ (data not shown).

Additional experiments were conducted with excised roots to examine the effect of CrFC on iron acquisition from other siderophores and from inorganic iron. When oat roots were provided with 6 μM CrFC in uptake solutions containing 2 μM FC, FOB, RA, or initially soluble $FeCl_3$, iron uptake rates were significantly decreased when compared to uptake rates from solutions without CrFC (Table III). These data supported the results obtained with whole plants which showed the Cr-siderophore inhibited iron uptake from both inorganic iron and from siderophores. In excised root experiments, the high uptake rate from inorganic iron showed that initially soluble inorganic iron was taken up more rapidly than iron chelated by siderophores. However, there was no apparent role of plant exudates or phytosiderophores; inorganic iron was not taken up except when added immediately prior to immersion of plant roots in the treatment solution. Moreover, inorganic iron in solution without siderophore precipitated out of solution over the 3-h uptake period as determined by measurement of soluble radioactive iron in aliquots of the solutions sampled before and after plant uptake.

Table II. *Effect of 10 μM CrFC on Shoot Content of ^{55}Fe (10^3 dpm mg^{-1} dry weight) in Hydroponically Grown Oat Provided with 0.02 μM [^{55}Fe]FC or Initially Soluble $^{55}FeCl_3$*

Different letters indicate significant differences between treatments (P = 0.05) by Tukey's HSD.

Iron Source	− CrFC	+ CrFC
Ferrichrome	9.65 a	1.14 c
$FeCl_3$	6.40 b	0.30 d

Table III. *Effect of 6 μM CrFC on Iron Uptake Rate ($\mu g\ g^{-1}$ dry weight h^{-1}) from 2 μM Siderophores or Initially Soluble Inorganic Iron by Excised Roots of Iron-Stressed Oat*

Different letters indicate significant differences between treatments (P = 0.05) by Tukey's HSD.

Iron Source	− CrFC	+ CrFC
Ferrichrome	0.52 c	0.27 c
Ferrioxamine B	1.57 b	0.50 c
Rhodotorulic acid	2.10 b	0.59 c
$FeCl_3$	3.56 a	0.45 c

DISCUSSION

Our experiments confirmed that oat has a microbial siderophore-mediated iron transport system that functions under conditions which preclude extracellular dissociation of siderophores or the use of phytosiderophores. Oat acquired iron from all five of the microbial siderophores but had marked specificity for FOB and RA, which were better utilized than FC, FCA, or COP. Siderophore-mediated iron transport was enhanced by precondition to iron stress and appeared to involve induction of an active membrane transport system that was inhibited by dinitrophenol or sodium azide.

Qualitatively similar data were obtained with whole plants and excised roots. Iron uptake rates by excised roots were not commensurate with the shoot iron contents measured in studies with intact plants, suggesting that excised roots take up iron more rapidly than intact plants, or that roots may accumulate iron that is not translocated to the shoot. However, excised-root experiments were useful for comparing siderophores and characterizing iron uptake kinetics under precise conditions, since the siderophores were less subject to degradation or change in concentration over a 3-h period than in the 3-week studies with whole plants. Results showed that 5 μM FOB, the apparent K_m for FOB-iron transport by excised roots, was effective for allowing iron-sufficient growth by whole plants.

The basis for siderophore specificity in oat has not been examined but may be similar to siderophore recognition by *Streptomyces* (13). Bacteria and fungi employ siderophore transport systems and NADH:siderophore reductases that have varying degrees of specificity. *Rhodotorula pilmanae*, a fungus that produces RA, is unable to use FOB for iron transport (12). However, Cr-siderophore competition studies with the filamentous bacterium *Streptomyces pilosus* show that FOB, RA, and FC are utilized by a common siderophore receptor/transport system (13). Muller *et al.* (13) suggest that siderophore recognition by *Streptomyces* may be based on the octahedral coordination of iron at the metal binding center, the only common structural feature of these three compounds.

Results of our experiments showing that oat has specificity for FOB and RA are in agreement with data of Smarrelli and Castignetti (22) who reported specificity for reduction of these siderophores by cytosolic NADH:nitrate reductase obtained from squash cotyledon. However, it remains to be determined whether siderophores are transported across the plasmalemma of root cortical cells or are reduced on the cell surface. Experiments that demonstrated inhibition of iron transport by CrFC indicated that all of the siderophores were used by a common iron transport system, but that FOB and RA were more readily reduced. Moreover, the ability of CrFC to inhibit inorganic iron uptake suggested that the reduction site involved the ion channel/carrier protein for inorganic iron.

Further studies are required to determine whether CrFC inhibited iron uptake by poisoning the plant iron transport system or, as in microbial iron transport, competitively inhibited chelate utilization by sterically hindering the siderophore/iron transport proteins. In ongoing work (DE Crowley, unpublished data), we are examining specific siderophore binding to membranes from partially purified plasma membrane fractions isolated from iron-stressed and iron-sufficient roots and are attempting to identify a siderophore reductase associated with the plasma membrane of root cortical cells.

In this research, iron acquisition from siderophores was examined under the most extreme conditions that would occur in the root environment to determine whether oat could directly utilize microbial siderophores. Mechanisms of iron acquisition by plants have been extensively investigated, and it is evident that a number of other plant responses to iron-stress could influence iron availability under less extreme conditions. In soils, indirect use of siderophores might occur under chemical conditions that permit dissociation of chelated iron. This indirect use of siderophores may be especially important for 'iron-efficient' dicotyledonous plant species that have the ability to reduce the pH and redox environment of the rhizosphere (20). Even with monocots that have little or no ability to alter the rhizosphere, some iron may be provided by siderophore dissociation in microsites reduced by soil microbial activity and root respiration.

Recently, monocotyledonous grasses, including oat, have been shown to utilize plant-produced chelates, 'phytosiderophores,' for iron acquisition (21, 23, 25). Under the conditions of our experiments, we were unable to measure any solubilization of inorganic iron by phytosiderophores produced by whole plants supplied with inorganic iron. This suggested that phytosiderophores were either quickly degraded and did not accumulate in the nonsterile nutrient solutions or that oat produced very low quantities of phytosiderophores that were diluted in nutrient solution culture. Other researchers have shown that phytosiderophores may supply iron at much higher rates than microbial siderophores when compared at a low concentration, and differences in uptake rates from microbial and phytosiderophores have led to the assertion that phytosiderophores are more important in iron nutrition of monocot grasses (21). However, this conclusion may not be well founded since, at low concentrations, total iron may be more limiting than the rate of uptake. For example, at the 0.01 μM level of phytosiderophore used to demonstrate high rates of uptake by barley (21), approximately 200 L of nutrient solution would be required to provide sufficient iron for 1 g of plant tissue containing 100 μg iron g^{-1} plant dry weight.

In the rhizosphere, redox, pH, and the rates of solubilization of iron and other solid phase minerals ultimately establish conditions that govern the utilization of different sources of chelated iron by both plants and microorganisms. Phytosiderophores have high affinity for copper and zinc, which inhibit iron chelation (24, 25), and from theoretical and empirical studies (6) have been predicted to be unstable with iron in the presence of soil zinc and copper minerals. However, because such predictions are based on the attainment of equilibrium, theoretical stability diagrams must be used in conjunction with empirical measurements of extractable metal ions, mineral dissolution rates, and models of nonequilibrium conditions resulting from plant uptake and rate-limiting diffusion.

This study identified 5 μM FOB as a minimum effective concentration for growth of oat seedlings in our hydroponic culture system. This value is similar to the physiological concentrations used by microorganisms and is comparable to both the 10 μM concentration of RA that is effective for growth of iron-efficient tomato (11) and to concentrations of iron supplied by fertilization with synthetic chelates used in agriculture. Recent studies by Bossier and Verstraete (3) show these levels of siderophore may reasonably occur in soils. Using *in situ Arthrobacter* bioassays that eliminate problems associated with soil extraction, these researchers reported 70 μg kg^{-1} FOB equivalents in bulk grassland soils. Following soil amendment with sucrose and L-ornithine to simulate organic-substrate-enriched rhizosphere soil, siderophore levels increased to approximately 600 μg kg^{-1} soil, a value that converts to approximately 10 μM siderophore in solution at 10% soil moisture. Based on our results that showed plant specificity for different siderophores, it will be important to identify and determine the concentrations of individual hydroxamate siderophores and to examine plant use of catechol and *Pseudomonas*-type siderophores that may also influence plant iron availability in soils.

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