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Fusarinines and dimerum acid, mono- and dihydroxamate siderophores from *Penicillium chrysogenum*, improve iron utilization by strategy I and strategy II plants

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Abstract

Cucumber, as a strategy I plant, and Maize as a strategy II plant, were cultivated in hydroponic culture in the presence of a ferrated siderophore mixture (1 μM) from a culture of *Penicillium chrysogenum* isolated from soil. The siderophore mixture significantly improved the iron status of these plants as measured by chlorophyll concentration to the same degree as a 100-fold higher FeEDTA supply. Analysis of the siderophore mixture from *P. chrysogenum* by HPLC and electrospray mass spectrometry revealed that besides the trihydroxamates, coprogen and ferricrocin, large amounts of dimerum acid and fusarinines were present which represent precursor siderophores or breakdown products of coprogen. In order to prove the iron donor properties of dimerum acid and fusarinines for plants, purified coprogen was hydrolyzed with ammonia and the hydrolysis products consisting of dimerum acid and fusarinine were used for iron uptake by cucumber and maize. In short term experiments radioactive iron uptake and translocation rates were determined using ferrioxamine B, coprogen and hydrolysis products of coprogen. While the trihydroxamates revealed negligible or intermediate iron uptake rates by both plant species, the fungal siderophore mixture and the ammoniacal hydrolysis products of coprogen showed high iron uptake, suggesting that dimerum acid and fusarinines are very efficient iron sources for plants. Iron reduction assays using cucumber roots or ascorbic acid also showed that iron bound to hydrolysis products of coprogen was more easily reduced compared to iron bound to trihydroxamates. Ligand exchange studies with epi-hydroxymugineic acid and EDTA showed that iron was easily exchanged between coprogen hydrolysis products and phytosiderophores or EDTA. The results indicate that coprogen hydrolysis products are an excellent source for Fe nutrition of plants.

Introduction

Iron utilization by plants is characterized by two strategies (I and II) both of which are generally independent of microbial iron acquisition pathways. Although the literature contains several reports on direct interactions of plant roots with microbial siderophores (Crowley *et al.* 1988; Crowley *et al.* 1992; Bar-Ness *et al.* 1991) the existence of transport systems in plant cell membranes recognizing microbial siderophores has not be proven so far. This is in contrast to many reports of microbial siderophore utilization, where fungal siderophores, like ferrichromes and coprogens or bacterial siderophores like ferrioxamines are recognized by bacteria and fungi (Drechsel & Winkelmann 1997). On the other hand recent data show that iron from microbial siderophores can be utilized by plants via iron exchange reactions () or via Fe(III) reduction (Yehuda *et al.* 1996). Thus, the presence of natural and synthetic iron chelates in the vicinity of plant roots may lead to increased iron utilization by various plants. The present investigation describes another route of iron transfer from microbial sources to plants involving fungal dihydroxamate and monohydroxam-

ate siderophores which originate as precursors under iron limitation (Diekmann 1970; Jalal *et al.* 1986) or result from degradation by fungal esterases (Emery 1976). Iron transfer from mono- and dihydroxamate fungal siderophores to the plant iron transport systems represent a further important mechanism of iron nutrition in plants.

Material and methods

Plant material

Maize (Zea *mays* cv. Alice) and cucumber (Cucumis *sativa* cv. Chinesische Schlange) were germinated for 3 days in quartz sand, and transferred to aerated nutrient solution containing K_2SO_4 0.7 mM, KCl 0.1 mM, $Ca(NO_3)_2$ 2 mM, $MgSO_4$ 0.5 mM, KH_2PO_4 0.1 mM, $MnSO_4$ 500 nM, $ZnSO_4$ 500 nM, $CuSO_4$ 200 nM, $(NH_4)_6Mo_7O_{24}$ 10 nM and H_3BO_3 1 μM (maize) or 10 μM (cucumber), respectively. Iron was supplied as described below. The plants were grown for 11–14 d (uptake and reduction experiments) or 21 d (long term experiments) in 2.5 l containers with 10 plants per container. The nutrient solutions were replaced every 3 days. The plants were grown under controlled conditions (day/night 16 h/8 h, 23 °C/21 °C, relative humidity 70% and a light intensity of approximately 200 μE $m^{-2}s^{-1}$) in a growth chamber.

Fungal strain and growth conditions

The fungal isolate was identified as a *Penicillium chrysogenum* strain which is a common inhabitant of all kinds of soils. Cultures of *P. chrysogenum* were kept on yeast-malt-extract glucose (YMG) agar slants.

Iron chelates and siderophores

Desferrioxamine B was obtained as Desferal (Novartis, Basel, Switzerland). Coprogen was isolated from *Neurospora crassa* as described earlier (Wong *et al.* 1985). Triacetylfusarinine was isolated from an unidentified Penicillium strain. The siderophore mixture of *P. chrysogenum* was isolated as described and designated as hydroxamate mix 'Hmix'. Corpogen hydrolysis products were used after identification of dimerum acid and fusarinines by HPLC and electrospray mass spectrometry.

Hydroxamate production (Hmix)

Cultures of *P. chrysogenum* were maintained on YMG-agar for approximately 3 weeks to allow sporulation. The spores were harvested and transferred to 1 l Erlenmeyer flasks containing 250 mL of low-iron asparagine-media as described by Thieken and Winkelmann (1992). After 10 days of incubation (25 °C, rotary shaker 75 rpm), the mycelia were filtered off and the culture filtrate was ferrated by adding $FeCl_3$(0.1 mM). The brown hydroxamate containing solution was then passed through an Amberlite XAD-4 column. After rinsing the column with distilled water, hydroxamates were eluted with 50% methanol. The eluate was evaporated to dryness and the residue dissolved in distilled water. This solution was stored at −20 °C and was designated Hmix (hydroxamate mixture).

Determination of iron and hydroxamate concentration

As the culture filtrate contained a variety of mono-, di-, and trihydroxamates a direct photometric quantification was not possible. Therefore, the total hydroxamate concentration was determined by saturation of the culture filtrate with ferric iron using an Fe-binding capacity method.

Aliquots of the solution were shaken with increasing amounts of $FeCl_3$ for 1 h. Then the pH was adjusted to 6.5 with MES-buffer (2-(N-morpholino)ethane-sulfonic acid) and the samples were shaken for another hour. After filtration, the colored Fe complexes were measured spectrophotometrically at 440 nm and correlated to iron saturation. From the resulting scatter plot the iron concentration, expressed as trihydroxamate equivalents, was calculated.

HPLC-analysis

HPLC analysis was performed using a reversed phase column (Nucleosil C18, 5 μm, 3.6 × 250 mm, Grom, Herrenberg, Germany) on a Shimadzu HPLC system (Shimadzu, Duisburg, Germany) equipped with a gradient controller and automatic sampler. Chromatograms were recorded using a gradient (6–40% acetonitrile in water, containing 0.1% trifluoro acetic acid). The wavelength was set at 435 nm to monitor the ferric hydroxamate complexes and at 220 nm to analyse the total hydrolysis products.

Mass spectrometry

Electrospray mass spectra (ES-MS) and tandem electrospray mass spectra (ES-MS/MS) were recorded on a triple-quadrupole mass spectrometer API III equipped with a pneumatically assisted electrospray (ion spray) ion source (Sciex, Thornhill, Canada). Aqueous samples were injected by a syringe infusion pump at 5 μL min^{-1} and measured in positive mode (orifice voltage 80 V). Tandem mass spectra (ES-MS/MS) were obtained by collision induced dissociation (CID) of the molecular ions with argon as collision gas (50 KeV). On-line HPLC/electrospray mass spectrometry (HPLC-ES-MS) was performed by connecting an HPLC system (Applied Biosystem, Weiterstadt, Germany) to an electrospray interface using a Nucleosil C-18 column (100 $\times$ 2 mm, 5 mm, Grom, Herrenberg, Germany) and a gradient of acetonitrile in water (6–40%) within 20 min containing 0.1% trifluoroacetic acid and a flow rate of 200 μL min^{-1}.

Hydrolysis of hydroxamates

Hydrolysis of coprogen was performed with HPLC-pure coprogen and for comparison purposes also with pure triacetylfusarinine C, using the ammoniacal methanol degradation method (Jalal & van der Helm 1981). Hydrolyses were also performed with NH_3(15N)/water (1:1) mixtures in order to obtain the fusarinines in their carboxylic acid forms.

Chlorophyll determination

Freeze-dried and ground plant material was homogenized and chlorophyll was extracted with 80% aceton. After centrifugation the supernatant was filtered and chlorophyll concentrations were determined spectrophotometrically using an extinction coefficient of 0.8993 l g^{-1} cm^{-1} at 652 nm.

^{59}Fe uptake experiments

Maize and cucumber plants were grown for 2 weeks in iron free nutrient solution. 12 h before uptake experiments the plants were transferred to 500 ml pots (1 plant per pot) containing a micronutrient-free solution. The uptake experiments were conducted in 150 ml beakers with micronutrient-free solution buffered with MES (5 mM) at pH 6.0 and supplemented with 1 μM radiolabeled Fe chelates for 5 h. Following, the shoots were cut to stop translocation and extracellular Fe was removed with bipyridyl and sodium dithionite as described by Bienfait *et al.* (1985). Roots and shoots were weighed after oven drying at 70 °C. ^{59}Fe activity was measured by liquid scintillation counting after ashing roots and shoots and dissolving the residues in 10% HCl.

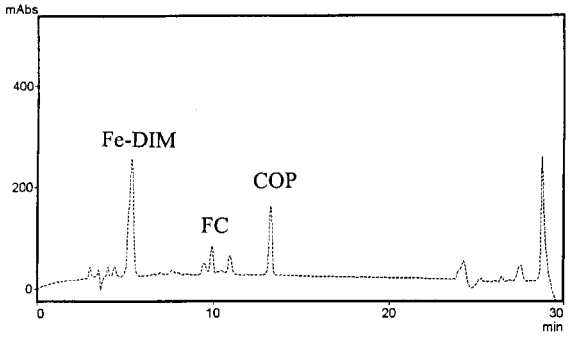

Figure 1. HPLC separation of siderophores isolated from a low-iron culture filtrate of *Penicillium chrysogenum.* Siderophores were separated on a C18 reversed phase column using a gradient (6–40% within 20 min) of acetonitrile +0.1% trifuoroacetic acid as a solvent system and monitored at 435 nm wavelength. For conditions see Materials and methods. Peaks were identified as follows: Fe-DIM = Fe-dimerum complex, FC = ferricrocin and other ferrichromes, COP = coprogen.

Iron reduction by cucumber roots

Plants were precultured with Fe (FeEDTA 10^{-4} M) or without Fe for 11 days. For the reduction experiment the plants were transferred to 50 ml Erlenmeyer flasks containing 20 ml of FeEDDHA, ferrioxamine B, FeHmix (5 μM each) or no iron substrate, bathophenanthrolinedisulfonate (BPDS, 15 μM) and MES-buffer (5 mM, pH 5.5) (Chaney, 1989, modified). Formation of the red Fe^{II} BPDS complex was quantified spectrophotometrically after 30 min, 1 h, 2 h, and 3 h.

Iron reduction by ascorbic acid

Ascorbic acid was added to solutions containing FeHmix or ferrioxamine B (40 μM each), MES-buffer (20 mM, pH 5.5) and BPDS (1 mM) to a final concentration of 20 mM. The formation of Fe^{II} BPDS complexes was measured spectrophotometrically at 534 nm over time.

Ligand exchange

Fe hydroxamate solutions (FeHmix or ferrioxamine B; final concentration: 200 μM) were mixed with free

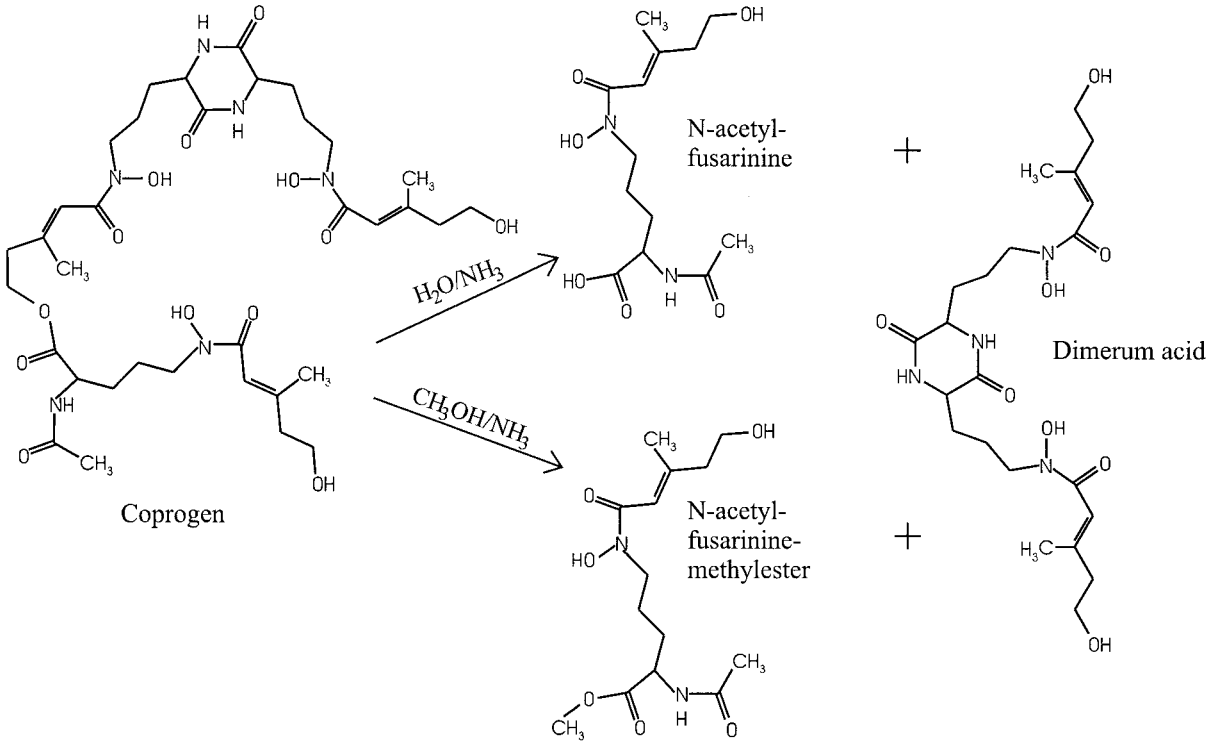

Figure 2. Structural formula of coprogen and its amoniacal hydrolysis products (iron-free).

ligands (*epi*-HMA or EDTA) at 2:1 molar ratio. The solutions were buffered with MES (20 mM, pH 6.0). The decrease of Fe hydroxamate concentration was measured during the experiment by its absorbance at 440 nm and compared to controls without free ligands. From these results the formation of FePS and FeEDTA was calculated.

Results

Analysis of siderophore production by the fungal isolate *P. chrysogenum* revealed that after 3 weeks of cultivation in shake cultures a mixture of different siderophores were present, e.g. coprogen and ferricrocin (trihydroxamates), dimerum acid (dihydroxamate) and fusarinines (monohydroxamates). Figure 1 shows an HPLC chromatogram of the original siderophore mixture from *P. chrysogenum*. Electrospray mass spectrometry confirmed the presence of coprogen and dimerum acid as the major siderophores. However, the composition of siderophores in the culture medium may vary, depending on the time of cultivation, medium constituents and pH. In general, prolonged cultivation resulted in larger amounts of ferrichrome type siderophores, such as ferricrocin. In order to study the plant growth effect of dihydroxamates and monohydroxamates, pure coprogen was hydrolyzed with ammonia either in methanol or in water (Figure 2). The hydrolysis products were analyzed by HPLC as shown in Figure 3. Due to the lower iron binding affinity of monohydroxamates only ferric dimerum acid (Figure 3 lower) could be detected in TFA-containing HPLC systems at 435 nm wavelength (Figure 3 middle). A complete analysis of hydrolysis products was obtained by monitoring the HPLC separation at 210 nm wavelength (Figure 3 upper). Three major peaks were seen in the chromatogram, representing N-acetylfusarinine (F), dimerum acid (D) and N-acetylfusarinine methylester (FME). Some byproducts have been obtained (U, P) which may represent condensation products, e.g., 1-hydroxy-3-acetylaminopiperidone (P) as described by Moore & Emery 1976.

Long term experiments with plants

Experiment 1

Cucumber plants as strategy I species were grown for 3 weeks in hydroponic culture with different iron

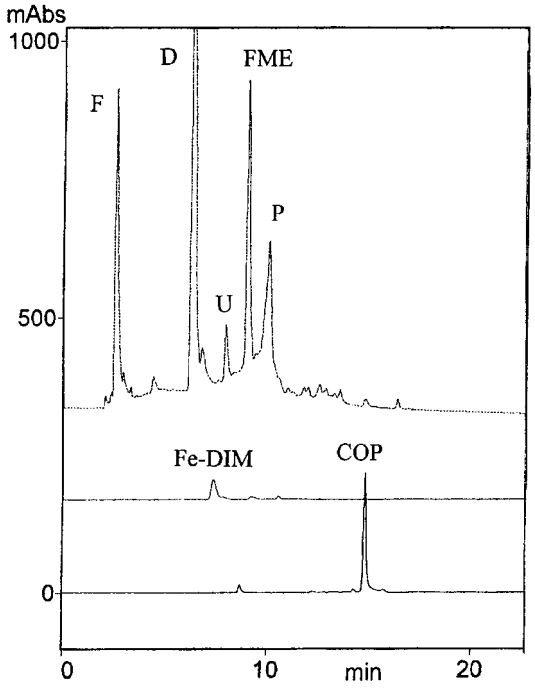

Figure 3. HPLC separation of coprogen hydrolysis products on a C18 reversed phase column using a gradient (6–40% within 20 min) of acetonitrile +0.1% trifuoroacetic acid as a solvent system monitored at a detector wavelength of 220 nm (upper) compared with a detection at 450 nm of the hydrolysis products (middel) and the original coprogen (lower). For conditions see Materials and methods. Identification of peaks were as follows: F = fusarinine, D = dimerum acid, FME = fusarinine methylester, P = piperidone condensation product, U = unknown, Fe-DIM = Fe-dimerum complex, COP = coprogen.

supply: 100 μM FeEDTA (generally used as +Fe control), −Fe and 1 μM FeEDDHA and FeHmix, respectively (Figure 4). The chlorophyll concentration of the two youngest leaves was used as a parameter for Fe status of the plants. No significant differences could be found between FeEDTA and FeHmix treatments although Fe concentration in the nutrient solution differed by factor 100 (Table 1). Plants supplied with FeEDDHA showed a slight decrease in chlorophyll content.

Experiment 2

Maize was grown without Fe and with FeEDTA, FeHmix and ferrioxamine B as Fe sources in various concentrations and the chlorophyll content of the two youngest leaves was determined after 3 weeks (Table 1). No chlorosis occurred when iron was supplied as EDTA or Hmix in high concentrations of 100 μM and 10 μM respectively. In low iron treatments chlorophyll concentrations were higher when iron was supplied as Hmix (0.1 μM) than as FeEDTA (1 μM). Compared to the control (−Fe), iron provided as ferrioxamine B had no effect on chlorophyll content even in higher concentration (10 μM).

Short term experiments

Uptake and translocation of ^{59}Fe

In these experiments Fe uptake and translocation rates were compared using the trihydroxamate siderophores ferrioxamineB and coprogen, coprogen hydrolyzates containing Fe-dimerum acid, N-acetylfusarinine (FSRN) or N-acetylfusarinine methylester (MFSRN), the mixture of hydroxamates produced by *P. chrysogenum* (mainly coprogen hydrolysate) and EDDHA. Both, strategy I (cucumber) and strategy II (maize) plants were tested. Figure 5 shows the results of 4 (cucumber) and 2 (maize) experiments, respectively. Values are expressed as per cent of Fe uptake and translocation from FeHmix. The absolute iron uptake and translocation rates [μmol Fe/g root d.m.] differed between the experiments, due to variation of iron-stress-response at the date of the experiment and due to different physiological age or contamination of the nutrient solution during preculture.

Ferrioxamine B provides only negligible amounts of iron to the plants while uptake and translocation from coprogen in both, cucumber and maize, was intermediate. All coprogen hydrolysates (MFSRN, FSRN, Hmix) were as effective as EDDHA when supplied to cucumber. Uptake and translocation rates in maize were very similar to those found in cucumber although the availability of iron supplied as EDDHA was very low and uptake was higher when iron was provided as the naturally formed hydrolysate (Hmix) compared to the NH_4 hydrolysates MFSRN and FSRN.

Examination of strategy I mechanism

Iron reduction by cucumber roots

Iron sufficient and deficient cucumber plants were transferred to solutions containing different Fe^{3+} sources. Fe deficient plants reduced only small amounts of Fe when supplied as ferrioxamine B (Figure 6). Higher Fe^{2+} concentrations were detected in FeEDDHA containing solutions and the highest reduction rate was found in FeHmix treatments. No differences between the Fe chelates could be found when supplied to iron sufficient plants which were

Figure 4. Growth of cucumber in the presence of different iron chelates and a ferric hydroxamate mixture from a culture of *Penicillium chrysogenum*.

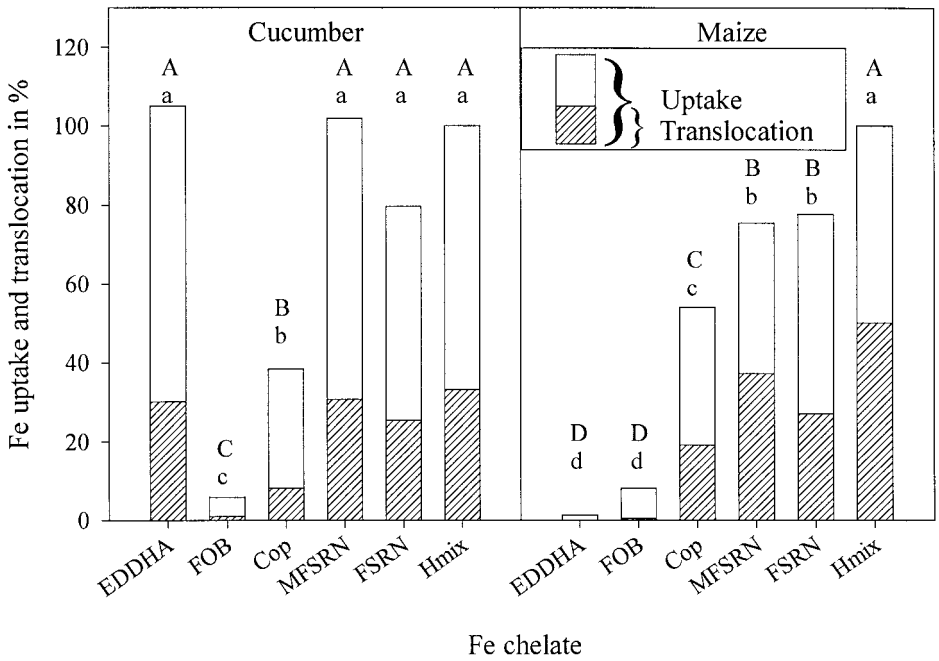

Figure 5. Iron uptake and translocation rates of cucumber and maize supplied as different iron chelates. Values are means of 4 (cucumber) and 2 (maize) experiments, respectively and are represented as percent of the fungal ferric hydroxamate mixture (FeHmix = 100%). Different letters indicate significant differences at p = 0.05. Total columns = uptake; streaked columns = translocated part.

Table 1. Chlorophyll content [mg g^{-1} dry wt.] of the two youngest leaves of cucumber and maize grown with different iron chelates for 3 weeks. n.d. = not determined.

Fe-concentration [μM]	EDTA	Hmix	Fe-chelate		Hmix	FOB
			EDDHA	EDTA		
		Plant species				
		Cucumber			Maize	
100	14.1 ± 1.8	n.d.	n.d.	13.8 ± 1.6	n.d.	n.d.
10	n.d.	n.d.	n.d.	12.7 ± 1.7	11.8 ± 1.6	2.2 ± 0.1
1	n.d.	13.1 ± 0.8	10.0 ± 1.1	4.4 ± 0.6	8.3 ± 0.6	n.d.
0.1	n.d.	n.d.	n.d.	n.d.	5.2 ± 0.7	n.d.
−Fe		1.7 ± 0.6			2.5 ± 0.3	

precultured with 100 μM FeEDTA. The amount of reduced iron is higher than in the −Fe/ferrioxamine B (preculture/substrate) treatment, even without addition of any iron. This indicates that apoplasmic iron and/or iron precipitated on the root surface can be reduced more easy than ferrioxamine B. Similar results were obtained with maize and barley: the standard reductase reduced more extraplasmatic iron (FeEDTA 10^{-4} M/no substrate) than FeEDTA (-Fe/FeEDTA 10^{-4} M) (Hördt, unpublished data).

Iron reduction by ascorbic acid

The reduction kinetics of ferrioxamine B and FeHmix expressed as% of total Fe using ascorbic acid as reductant was measured at pH 5.5. After 7 h more than 90% of both Fe chelates were reduced (data not shown). The reduction of FeHmix, however, occurred much more rapidly. Fe^{2+} concentration obtained by reducing FeHmix was twice as much compared to ferrioxamine B after 10 min. and still one and a half times higher after 1 h.

Examination of strategy II mechanism

Ligand exchange

The formation of Fe-*epi*-hydroxymugineic acid (PS) and FeEDTA using either FeHmix or ferrioxamine B as iron source is represented in Figure 7. From the latter neither the phytosiderophore nor the synthetic chelate obtained iron in considerable quantities. A higher ligand exchange rate was observed when Fe was supplied as FeHmix. Under the chosen experimental conditions, in less than 2 h about 1/3 of PS and 2/3 of the EDTA molecules chelated iron, removed from the hydroxamate complex.

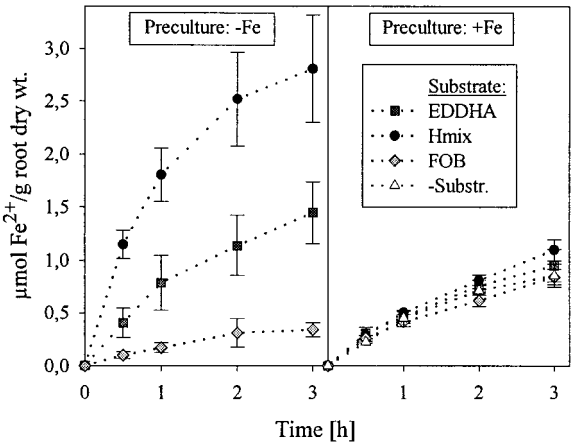

Figure 6. Iron reduction rates of cucumber roots after various pretreatments (+/− Fe) and supplied with different iron chelates. Values are expressed as μmoles Fe^{2+} per g root dry weight.

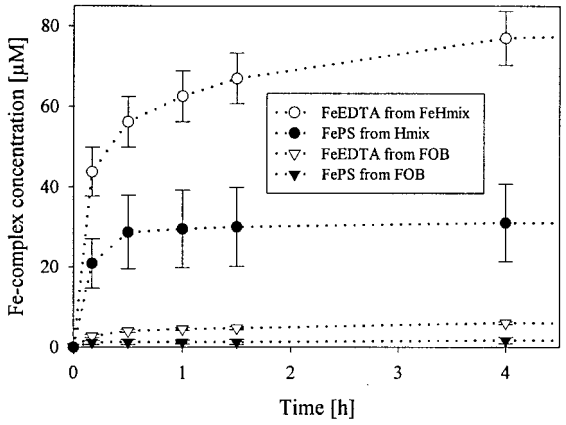

Figure 7. Ligand exchange rates from the fungal ferric hydroxamate mixture (FeHmix) or ferrioxamine B (200 μM) to phytosiderophore (epi-hydroxymugnineic acid) or EDTA (100 μM). Formation of the colorless FePS and FeEDTA complexes was calculated from the decline of iron hydroxamate complexes measured at 440 nm.

Discussion

The utilization of iron from microbial iron chelates by plants was the objective of many previous studies. In long term experiments under nonsterile conditions different results were obtained when iron was supplied as trihydroxamate complexes like ferrioxamine B or ferrichrome, depending on plant species or cultivar and experimental conditions. In sand culture, Römheld and Marschner (1986) found higher chlorophyll concentrations in the leaves of peanut than in maize with ferrioxamine B as iron source. Cline *et al.* (1984) obtained green sunflower in nutrient solution amended with ferrioxamine B (100 μM) whereas sorghum became chlorotic. The utilization of iron supplied as ferrichrome by oat as a strategy II plant species is described by Reid *et al.* (1984). The same cultivar remained green in nutrient solution containing 5 μM ferrioxamine B (Crowley *et al.*, 1988) while in the present experiments maize became chlorotic even when grown in 10 μM ferrioxamine B (Table 1). In these experiments microorganisms are an unpredictable factor. The inoculum is accidental and no investigations were made in which way these microorganisms act (e.g. metabolization of siderophores, degradation of root exsudates). Therefore, no conclusion could be made how the plants utilize iron from microbial siderophores. The determination of the uptake and translocation rate in short term experiments (1–6 h) gives more detailed information because microbial activity is lower and the leaf iron (translocation) is a fraction really taken up by the plant and not by root colonizing microorganisms or Fe precipitated on the root surface. High uptake/translocation rates may result from direct uptake of the Fe-complex via a specific uptake system (i.e. FePS uptake in strategy II plants), a high redox potential of the complex (Fe reduction by strategy I plants), or a low stability constant of the complex (ligand exchange by strategy II plants) Generally, the published uptake/translocation rates of Fe supplied as stable trihydroxamate siderophores are low compared to other Fe chelates like FeEDTA (strategy I plants) or FePS (strategy II plants) (Römheld 1987, 1991; Zhang *et al.* 1991; Crowley *et al.* 1992; Bar-Ness *et al.* 1991, 1992). These findings are in agreement with the results we obtained by maize and cucumber when iron was supplied as ferrioxamine B (Figure 5). For this reason it must be concluded that in the tested plant species no specific uptake system for trihydroxamate type siderophores exists. As shown in Figures 5 and 6 and as described in the literature (Römheld 1983, 1987; Bar-Ness *et al.* 1991; Yehuda *et al.* 1996) also reduction rates and ligand exchange rates (with EDTA and PS) of ferrioxamine B are low. This can be explained by the low redox potential ($E_0 = -470$ mV, Raymond *et al.* 1984) and the high stability constant ($\log ß_{110} = 30.6$, Crumbliss 1991) of the iron complex.

The utilization of iron from stable ferrichromes and ferrioxamines by plants in long term experiments seems to be the result of microbial activity. Two ways to rise iron availability are conceivable:

(1) Uptake of the complex by root colonizing microorganisms and remobilization (reduction or chelation) of the incorporated iron after death and decomposition of the microbial cells. In short term iron uptake experiments with ferrioxamine B Crowley *et al.* (1992) found 10–20-fold higher iron uptake (= Fe in shoots, roots and root adhering microorganisms) in inoculated than in non inoculated treatments but a lower translocation rate. It was concluded that iron was taken up mainly by microorganisms and not by the roots themselves. Iron uptake from hydroxamate type siderophores by microorganisms is evident, however, little is known about the subsequent utilization of this iron by plants after lysis of the microorganisms.

(2) Microbial degradation of the hydroxamate ligand. Hydroxamate degrading microorganisms are known since long time. Warren and Neilands (1964, 1965) isolated the ferrichrome A and ferrichrome metabolizing Pseudomonas strain FC-1. In 1990, a Gram-negative bacterium, DFBC 5, was isolated which grows on media with desferrioxamine B as sole carbon source (Castignetti and Siddiqui). A desferrioxamine B and E degrading *Azospirillum irakense* (ASP-1) is described in Winkelmann *et al.* (1996, 1999). In most cases the products of these metabolisms are di- or monohydroxamic acids with a lower affinity to iron.

Redox potential and stability constant of the ferric coprogen complex are similar to ferrioxamine B (Crumbliss 1991). But the chemical structure of coprogen (Figure 2) offers an alternative way to increase plant availability by splitting the ester-bond. Fe complexes of the resulting dihydroxamate (dimerum acid, a dimer of *trans*-furarinine) and monohydroxamate (*N*-acetyl-fusarinine) are less stable. Iron uptake uptake and translocation rates of coprogen hydrolysis products are much higher in both, strategy I and strategy II plants, than of ferrioxamine B and higher than of the trimer (Figure 5). Responsible for the enhanced Fe uptake is not a specific uptake system of the plant for microbial siderophores but a higher re-

duction rate (strategy I plants, Figure 6) and ligand exchange rate (strategy II plants, Figure 7). No differences in iron uptake and translocation rates were found between aqueous and methanolic ammoniacal hydrolysates. Slightly higher values were measured in maize when iron was supplied as the natural siderophore mixture (Hmix) compared to the NH_3-hydrolysates (FSRN and MFSRN) indicating that the ratio of mono and dihydroxamates may also be important. NH_3-hydrolysis yielded both, the fusarinine monomer and the dimer dimerum acid. Dihydroxamates like dimerum acid are known to have a higher stability compard to the monohydroxamic fusarinines but redox potential seems to be similar. Because of the high uptake/translocation rates of all tested hydrolysis products it is supposed that mono- and dihydroxamates in general can provide iron in a highly available form to plants. The Fe-dimerum complex may exist as a dimer ($Fe_2(DA)_3$) in neutral solution or as a monomer ($FeDA^+$) in acidic solution. A mixed ferric complex of fusarinie and dimerum acid (molar ratio 1:1) may also be possible (Jalal *et al.* 1986) giving rise to a variety of different structural, physical and chemical species at the root surface. On iron limiting calcareous soils it is expected that ester-containing siderophores like coprogen or fusigen are not very stable and will eventually be split in plant available mono- and dihydroxamates. A similar conclusion has been drawn from investigations about metabolisms of plant-protective agents. In alkaline soils e.g. Phenmedipham (methyl-(3-hydroxyphenyl)-carbamate-3-methylphenyl-carbamate-ester) is detoxified via hydrolysis. Other herbicides like Clorphenprop-methyl become effective after hydrolysis of the ester linkage in the soil (Müller 1986). Contrary to ferrioxamines and ferrichromes which are highly resistant to degradation and are metabolized by only few very specific microorganisms, the hydrolysis of ester linkages in fungal siderophores seems to occur more easily and is widespread among fungi in soils. The fact, that dimerum acid and fusarinine accumulate during prolonged cultivation by the producing fungus may suggest that an esterase is produced by the fungus which results in different breakdown products being released into the media, although the occurence of the esterase as an external or internal enzyme has not been determined in the present investigation. Accumulation of mono and dihydroxamates in the coprogen-producing *P. chrysogenum* may also result from overproduction of precursors as has been shown in *Gliocladium virens* by Jalal *et al.* (1986). Irrespective of their origin, the present investigation is evidence that monohydroxamtes and dihydroxamates can play an important role in increasing iron availability for plants grown on soils with low iron availability.

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