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Mössbauer study of iron uptake in cucumber root

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Abstract ^{57}Fe Mössbauer spectroscopy was used to study the uptake and distribution of iron in the root of cucumber plants grown in iron-deficient modified Hoagland nutrient solution and put into iron-containing solution with 10 μM Fe citrate enriched with ^{57}Fe (90%) only before harvesting. The Mössbauer spectra of the frozen roots exhibited two Fe^{3+} components with typical average Mössbauer parameters of δ=0.5 mm s^{-1}, Δ=0.46 mm s^{-1} and δ=0.5 mm s^{-1}, Δ=1.2 mm s^{-1} at 78 K and the presence of an Fe^{2+} doublet, assigned to the ferrous hexaaqua complex. This finding gives a direct evidence for the existence of Fe^{2+} ions produced via root-associated reduction according to the mechanism proposed for iron uptake for dicotyledonous plants. Monotonous changes in the relative content of the components were found with the time period of iron supply. The Mössbauer results are interpreted in terms of iron uptake and transport through the cell wall and membranes.

Key words iron uptake · iron(III) reduction · plant nutrition · cucumber (*Cucumis sativus* L.) root · Mössbauer spectroscopy

1 Introduction

Nowadays, the transport and storage of metal ions (including iron) in plants present a considerable scientific interest, since plants can accumulate trace elements and, in

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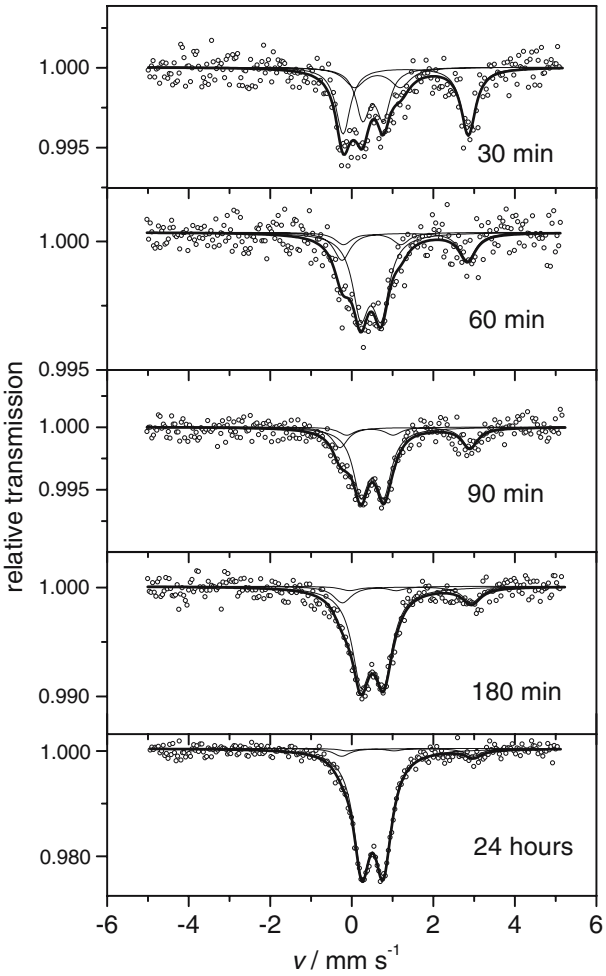

Figure 1 Mössbauer spectra, recorded at 78 K, of roots of cucumbers grown in iron-deficient nutrient solution and which were supplied with iron only before harvesting for times indicated in the figure (*on the left*).

particular, heavy metals. Thus plants are a potential intermediate "reservoir" for trace elements originated from the lithosphere, hydrosphere and atmosphere [1–3]. This is of importance for plant nutrition studies and phytoremediation-based biotechnology.

Some recent studies [2, 3] of metal transport in cucumber indicated significant differences for different metals. Iron is considered as an important plant micronutrient; it is also a key metal in energy transformations needed for synthesis and other vital processes in cells.

Plants use two distinct strategies [4] to solubilize and absorb iron from soil. Plants either reduce Fe(III) complexes at the root surface and absorb the resulting Fe^{2+} ions produced via root-associated reduction (strategy group I), or excrete specific Fe(III)-binding, low-molecular-weight organic polydentate ligands known as phytosiderophores, which solubilize Fe^{3+} ions and make them available for absorption (strategy group II). These two strategies are used by two distinctly separate taxonomic groups of plants, with the phytosiderophore-based mechanism restricted to the grass family and the Fe(III) reduction mechanism restricted to the dicots and monocots not included in the grass family. The cucumber belongs to the latter group.

Table I Mössbauer parameters, recorded at around 78 K, of roots of cucumbers grown in iron-deficient nutrient solution which were supplied with iron only before harvesting for times indicated in the table

Sample characterisation		Fe(II) component D			Fe(III) A component DA			Fe(III) B component DB		
Time interval of iron supply	W mm s^{-1}	δ_1 mm s^{-1}	Δ_1 mm s^{-1}	A_1 %	δ_2 mm s^{-1}	Δ_2 mm s^{-1}	A_2 %	δ_3 mm s^{-1}	Δ_3 mm s^{-1}	A_3 %
30 min	0.42	1.35	3.19	48.0	0.49	0.46	32.0	0.52	1.11	20.0
60 min	0.46	1.31	3.05	22.9	0.46	0.44	60.0	0.45	1.15	17.1
90 min	0.47	1.30	3.19	22.6	0.50	0.50	68.7	0.44	1.14	8.7
180 min	0.49	1.35	3.17	15.6	0.50	0.48	81.7	0.50	1.14	3.9
24 h	0.48	1.37	3.23	6.4	0.51	0.52	91.0	0.47	1.12	2.5

^{57}Fe Mössbauer spectroscopy is a promising and useful tool to study the chemistry of Fe in plants. However, the iron content of plant tissues under normal nutrition conditions is insufficient for Mössbauer measurements. Previous Mössbauer investigations, performed with plants grown either at a high iron content of isotopically enriched nutrient solutions [5–7] or in an extremely acidic soil environment with a very high iron content [8], indicated only Fe(III) species in the roots of duckweed, stocks, pea [5, 6], and a perennial grass [8].

The aim of this work was to get information about the microenvironments and valence state of iron taken up by cucumber roots using Mössbauer spectroscopy.

2 Experimental

Cucumber (*Cucumis sativus* L.) seeds were germinated in complete darkness on wet filter paper at 26°C for 30 h. The seedlings were then kept on 0.5 mM $CaSO_4$ solution for 24 h before transferring to light and nutrient solution. The nutrient solution was a modified quarter strength Hoagland solution of the following composition: 1.25 mM KNO_3, 1.25 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.5 mM $MgSO_4{\cdot}7H_2O$, 0.25 mM KH_2PO_4, 11.56 μM H_3BO_3, 4.6 μM $MnCl_2{\cdot}4H_2O$, 0.19 μM $ZnSO_4{\cdot}7H_2O$, 0.12 μM $Na_2MoO_4{\cdot}H_2O$, 0.08 μM $CuSO_4{\cdot}5H_2O$. Iron was supplied as Fe(III) citrate at 10 μM concentration containing 90% enriched ^{57}Fe. Culture solutions were renewed three times a week and the pH was kept below 5.5. The plants were illuminated with 100 $\mu molm^{-2}s^{-1}$ ppfd for 12/12 h light/dark photoperiod. The temperature was 20/26°C night/day. Cucumbers were grown in 400-ml plastic pots. The plants were put into iron-containing solution only before harvesting for 0.5 to 24 h. After three weeks of growth the plants were harvested and the roots were immediately frozen to 78 K.

^{57}Fe Mössbauer spectra were recorded in the transmission geometry with a conventional Mössbauer spectrometer (Wissel). γ-rays were provided by a 3×10^9 Bq ^{57}Co/Rh source. The measurements were performed at between 74 and 250 K in a temperature-controlled cryostat (Leybold, Germany). Isomer shifts are given relative to α-iron. The Mössbauer spectra were analysed by the least-squares fitting using Lorentzian lines using the MOSSWINN code [9].

3 Results and discussion

Figure 1 shows the Mössbauer spectra, recorded at around the temperature of liquid nitrogen, of roots of cucumbers grown in iron-deficient nutrition solution and then kept in iron-containing solution for times between 30 min and 24 h immediately before harvesting.

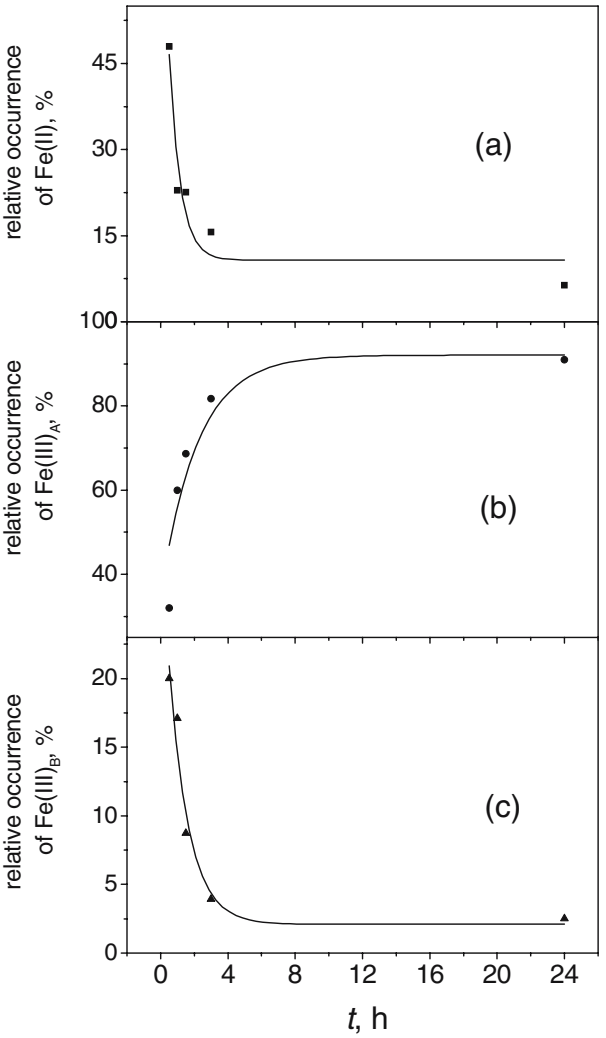

Figure 2 Time dependence of the relative content of components, Fe(II) (a), $Fe(III)_A$ (b) and $Fe(III)_B$ (c) (*on the right*).

The spectra were decomposed into three symmetrical doublets, D, DA and DB. The Mössbauer parameters are presented in Table I. The line width for all components remained around or below 0.5 mm/s even in the case of simultaneous fitting of the spectra. The doublets can be associated with three different iron microenvironments in those cucumber roots. Doublet D represents an Fe(II) species while doublets DA and DB are attributed to two different Fe(III) microenvironments denoted as $Fe(III)_A$ and $Fe(III)_B$. We have found monotonous changes in the relative content of the components with the time period of iron supply, as can be seen in Figures 1 and 2, as well as in Table I. The Fe(II) species, which is the dominant component when cucumber was kept for 30 min in the iron-containing solution, decreases exponentially to a very minor component after 24 h (Figure 2a). At the same time, the $Fe(III)_A$ component increases exponentially and reaches 91% after 24 h (Figure 2b). On the other hand, an exponential decrease of the $Fe(III)_B$ component occurs from 20 to 2.5% within the investigated time interval (Figure 2c).

A further strong confirmation of the correctness of the above spectrum decomposition was given by evaluating the spectra of those cucumber roots which had been kept in iron-sufficient solution when components DA and DB were also present but no ferrous iron could be identified. A detailed description and discussion of these results will be published elsewhere because of the limited size of this paper.

The Mössbauer results presented here can be interpreted in terms of iron uptake and transport through the cell wall and membranes. The component D is attributed to ferrous hexaaqua complex based on its characteristic Mössbauer parameters [10]. This species is expected to be formed from Fe(III) compounds outside the membranes of roots growing in the nutrient solution (or soil), in the case of plants belonging to strategy group I, via the mechanisms of active electron and proton transport through the membrane. The transported protons can enhance the solubility of iron at the cell wall, while the transported electrons reduce the Fe(III) to Fe(II) which can already enter into the cytoplasm. The transport mechanisms are accelerated in iron-deficient plants.

We have succeeded in showing by Mössbauer spectroscopy the presence of divalent iron in the plant root when the nutrient solution contained only Fe(III). This gives a direct evidence for the existence of Fe^{2+} ions produced via root-associated reduction according to the mechanism proposed for iron uptake in plants belonging to strategy group I. The changes in the relative content of the components shown in Figure 2 can indicate the transformation of Fe(II) hexaaqua complex into the $Fe(III)_A$ species. This agrees with the accepted viewpoint that the translocation and storage of iron inside the root cells take place in the form of Fe(III) compounds. The reaction rate of this Fe(II)-to-Fe(III) transformation is much higher than that of the Fe(III) reduction outside the membrane, when sufficient amounts of iron are taken up by the root, possible due to significantly lower Fe(II) reduction rate in these plants. Consequently, the Fe(II) species cannot be detected on the background of the Fe(III) components using Mössbauer spectroscopy in the case of iron-sufficient roots. The $Fe(III)_A$ component, which is the dominant and characteristic iron species in all cucumber roots, can be associated with an Fe(III) compound being in the cytoplasm (symplast) inside the cell. A spectral component similar to DA has already been found but not assigned in the case of rice root [7], too. The $Fe(III)_B$ species can be attributed to jarosite, $KFe_3(OH)_6(SO_4)_2$, or its analogous compound, since the Mössbauer parameters of DB agree with those of jarosite [11], and the nutrient solution contained suitable amount of sulfates. Moreover, jarosite has already been shown to be a major component in the rhizome of a perennial grass [8]. Jarosite is assumed to be loosely connected with the cell wall, which is supported by our observation that this component disappeared when the root was washed with EDTA which is supposed to mobilize the cell-wall-bound iron [3].

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