EFFECTS OF SHORT-TERM TREATMENT WITH IONIC AND CHELATED COPPER ON MEMBRANE REDOX-ACTIVITY INDUCTION IN ROOTS OF IRON - DEFICIENT CUCUMBER PLANTS

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Summary. The effects of different chemical forms of copper – ionic ($CuSO_4$) and chelated one [Cu(II)HEDTA], applied at micromolar concentrations, on the plasma membrane reductase activity (RA) and proton release in intact roots of cucumber plants grown hydroponically under iron deficiency were studied. Iron starvation provoked high induction of ferric-chelate reductase activity (substrates Fe(III)HEDTA and Fe(III)Citrate) and accelerated cupric-chelate reductase activity (measured with Cu(II)HEDTA and Cu(II)Citrate as electron acceptors), as well as hexacyanoferrate III RA (HCF III). Short-term application of cupric ions in the nutrient solution of iron-deficient plants resulted in a dramatic inhibition of Fe(III)HEDTA RA and Cu(II)Citrate RA and stopped H^+ release by intact roots. The reductase activity of iron-deficient cucumber roots, measured with HCF III, Fe(III)Citrate or Cu(II)HEDTA, however, was inhibited to a lower extent after cupric ions treatment. In addition, cupric-chelate Cu(II)HEDTA, applied at the same concentration in the nutrient solution of iron-deficient (–Fe) cucumber plants maintained the high stimulation of plasma membrane ferric-chelate RA, enhanced proton release by intact roots and produced additional acceleration of cupric-chelate RA and HCF III reduction in the roots. Application of cupric chelate Cu(II)HEDTA improved the iron-deficiency stress responses of cucumber plants.

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***Key words*:** cucumber (Cucumis sativus L.), iron deficiency, ionic and chelated copper treatment, ferric- and cupric-chelate reductase activity, hexacyanoferrate (III) reduction, proton release

Abbreviations: HCF III RA – hexacyanoferrate III reductase activity, Cu(II)Ch – and Fe(III)Ch RA – cupric-chelate and ferric-chelate reductase activity, HEDTA – [N-(2- hydroxyethyl) ethylenediamine triacetate]

INTRODUCTION

Plasma membrane-associated electron-transporting systems (or redox-systems) in plant cells have been involved in many processes directly or indirectly related to cell metabolism and tissue growth: transport of ions and small molecules, plasma membrane H^+-ATPase stimulation, changes in membrane energization and energy transduction, iron reduction and uptake in dicotyledonous and non-grass monocotyledonous plants, as well as in other processes including defence against pathogens and oxidative stress (Babalakova, 1992; Doering et al., 1998; Babalakova et al., 2003). Trans-plasma membrane redox reactions can participate in the establishment and maintenance of the apoplast redox status related to cell wall loosening and stiffening, thus influencing the interaction of various ionic or chelated compounds in soil or nutrient solutions. Among the plasma membrane reductases, ferric-chelate reductase activity induced by iron deficiency in roots of dicotyledonous plants is the best studied (Robinson et al., 1999; Schmidt, 1999; Moog and Brueggemann, 1994). Under conditions of adequate iron supply, intact roots of many dicotyledonous and monocotyledonous plants can also reduce different cupric-chelates, but their physiological roles and relation to copper uptake by plant roots are not clear, yet (Babalakova and Schmidt, 1996; Holden et al., 1996; Weger, 1999; Babalakova and Traykova, 2001). Resolving the regulation of iron and copper homeostasis in plant cells is extremely important for both plant productivity and human nutrition. Iron deficiency is spread in different crops, mainly in soils with high pH and increased calcium carbonate content (Romera et al., 1997; Wei et al., 1997). In response to iron deficiency, all plants except the grasses develop several biochemical and morphological reactions to ameliorate iron solubilization and uptake from the soil solution (Schmidt, 1999; Hell and Stephan, 2003). The biochemical and physiological mechanisms induced in dicotyledonous plants under conditions of iron deficiency comprise three main processes. The first one includes an increased release of protons through the activation of plasmalemma P-type ATPase proton pump to acidify the surrounding solution, thus enhancing Fe(III)-containing compounds solubility (Wei et al., 1997; Espen et al., 2000). The second process is an obligatory reduction of ferric-chelates by a membrane-associated Fe(III)-chelate reductase to the more soluble ferro-complexes (Chaney et al., 1972; Moog and Brьggemann, 1994; Robinson et al., 1999). The third

adaptive biochemical response is an induction of the synthesis of a specific transporter for ferro-ions in plasmalemma of root cells (Schmidt, 1999; Hell and Stephen, 2003). Besides a high induction of ferric-chelate reductase activity in roots of iron-deficient plants, the reduction capacity for cupric-chelates also rises, but its relation to the uptake of copper and iron is not clear (Babalakova et Schmidt, 1996; Holden et al., 1996; Babalakova and Traykova, 2001; Weger, 1999). It has been established that ionic copper causes an inhibition of both the induction and function of ferric-chelate reductase in roots of iron-deficient plants (Alcantara et al., 1994; Schmidt et al., 1997; Romera et al., 1997). However, no data concerning the influence of chelated copper on the iron-deficiency plant responses are available, offering us the possibility to compare the effects of ionic and chelated copper on the ferric- and cupric-chelate reductases in iron-deficient cucumber roots.

The reactivity of copper ions to form stable complexes and to participate in redox-reactions at the plasma membrane put forward the conception that copper can displace iron from Fe(III) complexes in nutrient solutions with iron supply (Guinn and Joham, 1963; Taylor and Foy, 1995; Laurie et al., 1991). Data describing what might be the plant reaction towards cupric-chelates in the absence of iron are not available. Some controversial data exist about the extent of uptake of ionic or chelated elements. It has previously been shown that accumulation of copper may be markedly affected by the chemical form of the applied copper depending on the charge of Cu-complexes. The comparison of the uptake patterns of positive copper complex with anion Cu-complex demonstrates that Cu(II)-EDTA is accumulated poorly (Coombes et al., 1977, 1978).

Recently, the results of Schmidt et al. (1997) underline that both ionic Cu and Cu(II)EDTA can be readily transported through the plasmalemma of root cells. The uptake of the intact chelate molecule has been reported to occur in chelator-buffering solutions indicated by increased Cu concentrations in plants grown in media with copper and other elements (Taylor and Foy, 1985; Bell et al., 1991; Laurie et al., 1991). The conclusions of some authors suggest that the primary toxic effect of Cu(II)EDTA could be the induction of iron deficiency in plant leaves (Taylor and Foy, 1985). The uptake of either ionic or chelated metals may depend on plant species or cultivar, stability and concentration of the metal complex and solution pH (Laurie et al., 1991).

The existing controversial results regarding copper effects on plant iron nutrition and various older data interpretations, as well as the experiments with high metal concentrations, stimulated us to compare the effects of micromolar concentrations of ionic and chelated copper on the activity of root membrane reductases and proton release in cucumber plants grown under conditions of iron starvation.

MATERIAL AND METHODS

Plant material

Seeds of cucumber (Cucumis sativus L. – cultivar Gergana) were germinated in Petri dishes on moistened with 0.1 mM $CaCl_2$ filter paper, in the dark at 28° C for 3 days. Uniform seedlings were put to grow in an environmental chamber in plastic pots on one-tenth concentration of Hoagland-Arnon I solution. Two days later the plants were divided into plus and minus Fe variants and fed on a half-strength nutrient solution, followed by complete nutrient solution. Nutrient solutions were changed every second day and pH was adjusted at 6.0 with KOH and supplemented with 20 μM Fe(III)-HEDTA (hydroxyethyl ethlenediamine-triacetic acid), prepared as Tris-KOH salt, pH 5.5 (for control, +Fe plants). Fe-deficient cucumber plants were grown on a nutrient solution without Fe.

Short-term treatment of control (+Fe) and Fe-deficient (-Fe) plants with ionic and chelated Cu

The first set of experiments was performed with 10-11-day-old cucumber plants (grown 5 days without Fe) and treated for 24 h with 10 or 20 μM $CuSO_4$, or 20 and 100 μM Cu(II)HEDTA. The plants were also treated with 20 μM Fe(III)HEDTA. The same procedure was repeated with 15-16-day-old plants (subjected to Fe-starvation for 10-11 days). The chemical forms of applied copper, used to compare their effects in control (+Fe) and Fe-deficient cucumber plants had different electrical charges. Ionic copper(II) sulfate pentahydrate forms in water solution cupri-hexahydrate cation - $[Cu^{II}(OH_2)_6]^{2+}$. HEDTA forms anion complex with copper sulfate in water solution – hydroxyethyl ethylenediamine-triacetato cuprate – $[Cu^{II}HEDTA]^{2-}$ (Coombes et al., 1978).

Assay of redox-proteins activity. Ferric- and cupric-chelate reductase activity (FeChRA, CuChRA) measurements

Two ferric-complexes - Fe(III)HEDTA and more natural Fe(III)Citrate, prepared as Tris-KOH salt stock solutions, pH 5.5 were used as enzyme substrates (electron acceptors). Cupric-chelate reductase activity was also measured with two copper complexes – Cu(II)HEDTA and Cu(II)Citrate (stock solutions with pH 6.5) to compare the rate of activity of both reductases. Intact cucumber roots reduction capacity was followed in dark vessels, 1 h duration as described previously (Babalakova and Schmidt, 1996, Babalakova and Traykova, 2001). The reductase activity of intact roots was expressed in μmol Fe(II) or Cu(I) / g R FW / h.

Hexacyanoferrate III (HCF III) was also used as impermeable electron acceptor

for evaluation of the activity of standard or constitutive redox-system at the plasmalemma of root cells. The reductase activity with HCF III was performed according to Schmidt (1994). The reductase activity was expressed in micromoles reduced iron per gram root FW per h.

pH change and proton release measurements

The change in the complete nutrient solution of (+Fe) and (-Fe)-cucumber plants was accompanied with daily pH monitoring in order to follow the expression of iron-deficiency responses as an enhanced proton release in the solution. To compare the effect of ionic and chelated copper on the proton release, several concentrations were used – 0.2 (control), 2 and 20 μM Cu^{2+} or equimolar concentrations of Cu(II)HEDTA. The changes in pH of the nutrient solution were followed in the course of three days.

Statistical analysis

The experiments were repeated at least 3 times with 6 to 8 intact plants in each variant. The data presented are the average of 18 to 24 samples and the values in the tables represented the standard errors of the mean values. The differences between the variants were compared by Student's t-test at 5% level of significance.

RESULTS AND DISCUSSION

Cucumber seedlings grown for 5 days without Fe in the nutrient solution started to develop leaf chlorosis and root morphological changes characteristic for iron deficiency. A marked enhancement of ferric- and cupric-chelate reductase activity (RA), and HCF III RA was also measured (Table 1 – A and B). We compared the reductase activity with two different electron acceptors, using Fe and Cu complexing agents– HEDTA and citrate. Fe-deficient cucumber roots demonstrated higher stimulation of ferric-chelate RA in the presence of Fe(III)HEDTA as an electron acceptor (about 7-fold) as compared with Fe(III)Citrate used as a substrate (about 4-fold increase as compared to the control, +Fe). Another difference included reactions of RA after short-term application of Cu^{2+}. Fe(III)HEDTA RA in iron-deficient cucumber roots was highly inhibited by ionic copper and became even lower than the control (+Fe) activity (Table 1–A and B). The considerable induction of ferric-chelate reductase activity due to iron starvation in cucumber roots disappeared entirely after copper ions treatment. The inhibition of RA in iron-deficient cucumber roots by copper ions with Fe(III)Citrate used as a substrate was lower and compared with the activity of (+Fe) plants some stimulation was registered (Table 1 – B). Iron starvation induced also a strong increase in the cupric-chelate reductase activity (about 7-8-fold), measured with Cu(II)Citrate as an electron acceptor, similar to the stimulation of

Table 1 –A and B. Effect of short-term treatment with ionic copper (20 μM Cu^{2+}) on the reduction rate of ferric- and cupric-chelates in intact roots of 10-11-day-old control (+Fe) and iron-deficient (-Fe) cucumber plants.

A Variants	Reduction of Fe(III) - and Cu(II)-chelates (μmol Fe(II) or Cu(I) g^{-1} FW h^{-1})					
	Fe(III)-HEDTA		Cu(II)-Citrate		HCF III	
	Value	% to + Fe	Value	% to + Fe	Value	% to + Fe
+Fe	0.496 ± 0,035	100	0.217 ± 0.011	100	1.133 ± 0.076	100
-Fe	3.548 ± 0.115	715	1.806 ± 0.095	832	4.670 ± 0.280	412
+Fe + Cu^{2+}	1.010 ± 0.061	204	0.375 ± 0.017	173	1.805 ± 0.093	159
-Fe + Cu^{2+}	0.127 ± 0.007	3.6 (% to -Fe)	0.068 ± 0.004	3.8 (% to -Fe)	1.229 ± 0.067	26.3 (% to -Fe)
		25.6 (% to +Fe)		37.3 (% to +Fe)		108.5 (% to +Fe)
B	**Fe(III)-Citrate**		**Cu(II)-HEDTA**			
	Value	**% to + Fe**	**Value**	**% to + Fe**		
+Fe	0.215 ± 0.011	100	0.101 ± 0.005	100		
-Fe	0.905 ± 0.050	421	0.286 ± 0.016	283		
+Fe + Cu^{2+}	0.275 ± 0.014	128	0.175 ± 0.011	173		
-Fe + Cu^{2+}	0.315 ± 0.016	34.8 (% to -Fe) 146.5 (% to +Fe)	0.111 ± 0.006	39 (% to -Fe) 110 (% to +Fe)		

Fe(III)HEDTA RA in (–Fe) plants (Table 1 – A). However, lower (only 2-fold) stimulation was observed under conditions of iron deficiency using Cu(II)HEDTA as an electron acceptor for cupric-chelate RA (Table 1- B). A very strong inhibition of Cu(II)Citrate RA in (–Fe) plant roots was registered after 24-h treatment with ionic copper, the extent of inhibition being similar to that of Fe(III)HEDTA reductase activity. In our study, Fe-chelate RA was measured at a pH optimum of 5.5 and Cu-chelate RA optimum was higher at more alkaline pH (6.5). It was supposed for dicotyledonous plants that the reduction of ferric- and cupric-chelates could be performed by one and the same membrane reductase (Welch et al., 1993). Later investigations confirmed the presence of various redox proteins at the plasma membrane that can act as cupric- and ferric-chelate reductases (Babalakova and Schmidt, 1996; Holden et al., 1996; Weger, 1999; Babalakova et al., 2003). The pH optima of the two reductases were also different. Other results suggested that ferric-chelate reduc-

Table 2. Changes in reductase activity using different electron acceptors in the roots of 16-day-old cucumber plants grown under iron deficiency conditions and after short-term application of copper ions (20 μM Cu^2 , 24 h).

Variants	Reduction of Fe(III)- and Cu(II)-chelates (μmol Fe(II) or Cu(I) g^{-1} FW h^{-1})					
	Fe(III)-Citrate		Cu(II)-Citrate		HCF(III)	
	Value	% to + Fe	Value	% to + Fe	Value	% to + Fe
+Fe	0.170 ± 0.082	100	0.150 ± 0.07	100	0.950 ± 0.06	100
-Fe	0.750 ± 0.055	441	1.312 ± 0.11	875	4.338 ± 0.35	457
+Fe + Cu^{2+}	0.205 ± 0.012	121	0.230 ± 0.03	153	1.236 ± 0.07	130
-Fe + Cu^{2+}	0.243 ± 0.016	32.4 (% to -Fe) 143 % to + Fe	0.064 ± 0.01	4.9 (% to -Fe) 43 % to + Fe	0.893 ± 0.06	20.6 (% to -Fe) 94 % to + Fe

tase activity in Fe-deficient plants ("turbo" reductase) measured at pH 5.5 might be different from the constitutive redox-proteins (Holden et al., 1996; Susin et al., 1996; Babalakova and Schmidt, 1996). High activation of cupric-reductase in the roots of Fe-deficient plants might be connected with an increased copper uptake to the same extent under iron starvation (Herbic et al., 1996). The direct connection between enhanced cupric-chelate reduction and increased copper content in plant roots, however, is not clear (Babalakova and Traykova, 2001).

Older cucumber plants, grown for 10-11 days without Fe kept the high stimulation of FeChRA, CuChRa and HCR III RA under conditions of iron starvation (Table 2). Hexacyanoferrate III (FeCN) reductase activity as an expression of a constitutive redox-system at the plasma membrane was as high as Fe(III)Citrate RA (Table 1-A, Table 2) under conditions of iron deficiency. Treatment with copper ions showed the same reactions of iron-deficient reductases in roots as in younger plants. The induction of both Fe-chelate RA and HCF III RA under iron starvation in different plants varied to a different extent due to enzyme heterogeneity (Schmidt, 1994; Lynnes et al., 1998). The considerable inhibitory effect of ionic copper on plant root reducing capacity upon Fe deficiency confirmed previously obtained results (Alcantara et al., 1994; Romera et al., 1997; Schmidt et al., 1997). The addition of the same micromolar concentrations cupric chelate in the nutrient solutions of iron-sufficient and iron-deficient plants demonstrated different reactions of plant reductases. The effects of short-term treatment of control and iron-deficient cucumber plants with equimolar concentrations of ionic and chelated copper on Fe(III)-HEDTA RA and HCF III RA are presented in Tables 3 and 4. Application of Cu(II)HEDTA produced some additional increase of ferric-chelate reductase activity in both control (+Fe) and iron-

Table 3. Effects of ionic copper or chelated Cu and Fe on the Fe(III) HEDTA reductase activity in cucumber plants grown under conditions with normal iron supply or iron deficiency.

Variants 24 h treatment with ionic and chelated Cu or Fe	Fe(III)-HEDTA Reductase Activity (μmol Fe(II) g^{-1} FW h^{-1})				
	+ Fe	% to + Fe	– Fe	% to – Fe	% – Fe / +Fe
Control	0.436 ±0.071	100	3.67 ± 0.211	100	842
+20μM Cu^{2+}	0.854 ±0.065	196	0.13 ± 0.011	3.5	15
+20μM Cu(II)-HEDTA	0.580 ±0.031	133	4.49 ± 0.322	122	774
+20μM Fe(III)-HEDTA	0.685 ±0.032	157	3.92 ± 0.285	107	572

Table 4. Alteration of HCF III reductase activity in roots of cucumber plants after treatment with ionic or chelated Cu and Fe in control (+Fe) and iron-deficient (-Fe) cucumber plants.

Variants 24 h treatment with ionic and chelated Cu or Fe	HCF III Reductase Activity (μmol Fe(II) g^{-1} FW h^{-1})				
	+ Fe	% to + Fe	– Fe	% to – Fe	% – Fe / +Fe
Control	1.10 ± 0.065	100	4.84 ± 0.355	100	440
+20μM Cu^{2+}	1.55 ± 0.091	141	1.23 ± 0.081	25	79
+20μM Cu(II)-HEDTA	1.32 ± 0.084	120	7.13 ± 0.376	147	540
+20μM Fe(III)-HEDTA	1.34 ± 0.092	122	4.92 ± 0.343	102	367

deficient (–Fe) plants (Table 3). Thus, treatment with cupric chelates can keep the physiological response of iron deficient plants to develop very high reductase activity. A similar stimulation of HCF III RA was observed after Cu-chelate treatment (Table 4 – 5-fold increase of RA). To follow the induction of reductase activity in Fe-deficient cucumber plants we carried out treatment with Fe(III)HEDTA as a source of iron nutrition also (Tables 3 and 4). Ferric-chelate application for 24 h did not cause any decline of RA in iron-deficient cucumber roots, thus indicating that initial adaptation of cucumber plants after iron re-supply with ferric-chelate needed more than one day. We tested also the effect of a higher concentration of cupric chelate (100 micromoles) on the RA (Table 5). Cupric chelate application stimulated the Fe(III)HEDTA RA and HCF III RA in both control and iron-deficient plants. The alteration of RA in Fe-deficient plants was related to pH changes in the nutrient solutions during iron starvation and copper treatment. Application of ionic copper (Cu^{2+}) inhibited the release of protons by the roots of Fe-deficient plants (Fig. 1 – data with 2 and 20 μM Cu^{2+}) which was in correlation with the high inhibition of Fe-chelate RA by ionic copper. At the same time chelated copper application stimulated the H^+ extrusion by the roots of Fe-deficient cucumber plants and thus the pH of the

Table 5. Influence of short-term (24-h) application of cupric chelate in the nutrient solution on the ferric-chelate and hexacyanoferrate III reductase activities in the root plasma membrane of control (+Fe) and iron-deficient (-Fe) 15-day-old cucumber plants.

Variants	Reduction of Fe(III) - chelates (μmol Fe(II) g^{-1} FW h^{-1})			
	Fe(III)-HEDTA RA		HCF(III) RA	
	Value	% to + Fe	Value	% to + Fe
+Fe	0.340 ± 0.051	100	0.916 ± 0.048	100
−Fe	3.138 ± 0.154	923	4.788 ± 0.227	523
+Fe + 20 μM Cu(II) HEDTA	0.446 ± 0.032	131	1.079 ± 0.062	118
+Fe + 100 μM Cu(II) HEDTA	0.548 ± 0.035	161	0.989 ± 0.055	98
−Fe + 20 μM Cu(II) HEDTA	4.490 ± 0.283	143 (% to -Fe)	7.132 ± 0.357	149 (% to -Fe)
−Fe + 100 μM Cu(II) HEDTA	4.053 ± 0.294	129 (% to -Fe)	6.500 ± 0.323	136 (% to -Fe)

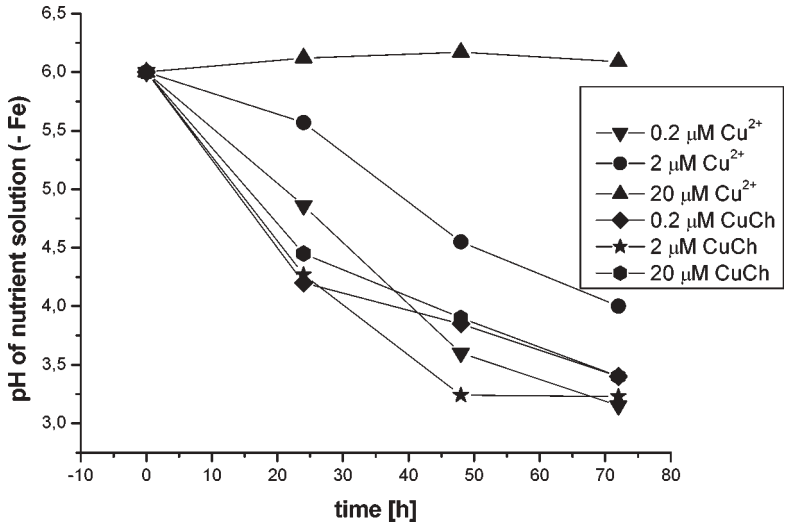

Fig. 1. pH alterations of the nutrient solution (initial pH 6.0) of iron-deficient (-Fe) cucumber plants grown in the presence of cupric ions or Cu-chelates [Cu(II)HEDTA] applied at micromolar concentrations.

nutrient solution of (–Fe) plants decreased from 6.0 to 4.2 - 4.3 after 24 h (Fig.1 – data with 2 μM Cu(II)HEDTA and 20μM Cu(II)HEDTA). Enhanced acidification of the medium during iron starvation is important for the induction and sustaining of the high level of ferric chelate RA in many plants because the enzyme is pH sensitive (Wei et al., 1997; Schmidt, 1999). It has recently been proved that high apoplastic pH depressed Fe-chelate RA and restricted the uptake of Fe(II) into the cytosol (Kosegarten et al., 2004). The applied concentrations of ionic and chelated copper had an insignificant effect on pH changes of the control (+Fe) solutions (data not shown). Our results showed a correlation between the proton release and the stimulation of ferric-chelate reductase activity under conditions of iron starvation. It has been suggested that the rate of cupric reduction is a function of the free Cu^{2+} as the actual substrate of cupric reductase activity (Holden et al., 1996; Weger, 1999). Our results showed that cupric-chelate reductase at the plasma membrane of Fe-deficient cucumber roots demonstrated a higher activity in the presence of chelated copper. One possible explanation for the inhibitory effect of ionic copper on FeCh RA in iron-deficient roots is based on our assumption that Cu^{2+} might act as a powerful scavenger of the superoxide radical, facilitating Fe-chelate reduction at the plasma membrane (Cakmak et al., 1987; Macri et al., 1992). This suggestion is supported by the experiments with *in vitro* application of copper ions that produced the inhibition of Fe(III)EDTA RA in (–Fe) plants already within the first minutes (Schmidt et al., 1997). Another effect of ionic copper inhibiting to a higher extent the proton release in (–Fe) cucumber plants, might be the reduced activity of the plasmalemma proton pump (Babalakova and Hager, 1994).

Ionic copper can also affect Fe nutrition by the inhibition of some components or subunits of the trans-plasma membrane electron transport chain. In the presence of chelating agents copper ions form chelates that can be uptaken by plant roots (Schmidt et al., 1997). These authors showed that at equimolar Fe and Cu levels, i.e. in the presence of Cu chelates, an induction of the iron-stress response was not inhibited, thus supporting our results for stimulation effects of cupric chelates on the FeChRA in Fe-deficient cucumber plants. The exact site of the copper action remains to be established. In spite of the intensive research, the role of chelation itself and metal complexing agents with different charges in the mechanisms of metal uptake by plants and their action on membrane level with the induction of reductase activity are not yet properly understood. Our results pointed out different levels of ferric- and cupric-chelate reductase activity with different ligands during iron starvation of cucumber plants. Ferric-citrate is known to be the more natural substrate, but the most common chelating agent used to enhance the water solubility of iron in hydroponics, is EDTA or a related substance, such as HEDTA. The high increase in the standard redox-system activity (HCF III RA) in Fe-deficient cucumber roots upon application of cupric chelates suggested strong enhancement of trans-membrane electron transport that might be connected with the sustained activity of the proton pump. Interactions

between the H^+-ATPase activity and the redox state of the cytoplasm have been suggested to play an important role in the regulation of electron transport by the standard redox system (Schmidt, 1999). Increased activity of standard reductase in iron-deficient plant roots in the presence of cupric chelates might be connected with its probable role for root morphology improvement under conditions of iron starvation (data not shown). Further research is needed to clarify the action of ionic and chelated copper at the membrane level in control and iron-deficient cucumber plants.

CONCLUSION

In the present study, we demonstrated that micromolar concentrations of cupric-chelate Cu(II)HEDTA, applied in the nutrient solution of iron-deficient cucumber plants maintained the stimulation of plasma membrane ferric-chelate reductase activity, as well as accelerated hexacyanoferrate III RA, and cupric-chelate reduction by intact plant roots. In addition, application of cupric chelate improved other stress responses under conditions of Fe-starvation, such as root morphology changes and enhanced proton release in the nutrient solution. These markedly expressed positive effects of cupric chelates were in contrast to the strong inhibitory action of copper ions applied at the same concentrations in the nutrient solutions of iron-deficient plants.

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