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Relationship of iron deficiency and the altered thylakoid development in Cd treated poplar plants

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Introduction

Multiple inhibitory effects of Cd on photosynthesis as leaf chlorosis, changes in chloroplast ultrastructure, inhibition of photosynthetic electron transport and the carbon reduction cycle have been observed (Krupa and Baszynski 1995). However, only few data are available on how the amount, ratio and structure of chlorophyll containing complexes determining photochemical and light-harvesting/dissipation efficiency in thylakoids are affected (Krupa 1988, Tziveleka *et al.* 1999, Sárvári *et al.* 1999). Strong interaction between Cd and iron metabolism has been observed (Siedlecka and Krupa 1999) which may play important role in the biogenesis and functioning of the photosynthetic apparatus. Therefore, we investigated the effects of Cd treatment and iron deficiency on the organisation of chlorophyll (Chl)-proteins as the function of iron content in leaves to see the possible relationship between these stresses.

Materials and methods

Micropropagated poplar plants (*P. glauca*, var. *Astria* and *P. alba*) were grown hydroponically in Hoagland solution of ¼ strength with 10 µM Fe-EDTA or Fe-citrate as a Fe supply. Four-week old plants already developed 4 leaves (negatively numbered downward from the level of treatment) were treated with 10 µM Cd(NO₃)₂ or by withdrawing the Fe supply up to six-week age during which they developed another 4-5 leaves (positively numbered upward from the level of treatment). Chl content (µg cm⁻² leaf area) and Chl-proteins (µg Chl cm⁻² leaf area) solubilised by mainly glucosidic detergents, separated by Deriphat PAGE, and identified on the basis of their polypeptide patterns were determined according to Sárvári *et al.* (1999). Fluorescence induction parameters were measured with a PAM fluorimeter (Walz). ¹⁴CO₂ fixation was studied according to Láng *et al.* (1985). Metal content and carotenoids were determined by total reflexion X-ray fluorescence spectrometry (TXRF) and HPLC, respectively. Data from two independent experiments were used.

Results

Fe deficient and Cd treated poplar plants showed reduced growth and, in addition, decreased Chl content. The symptoms *P. alba* and *P. glauca* did not differ significantly. Lowering of the Chl content, which is related to the strength of stress in the photosynthetic apparatus, was tightly connected with the decreased Fe content of the leaves both in Cd treated and Fe deficient plants (Fig. 1). While the Mn content was also lower in the leaves of Cd treated plants it increased in Fe deficient ones. The symptoms of Cd treatment were somewhat milder in Fe-EDTA than in Fe-citrate grown plants, and the Fe-citrate grown plants were hardly Fe deficient in opposition to Fe-EDTA grown ones (see Table 1).

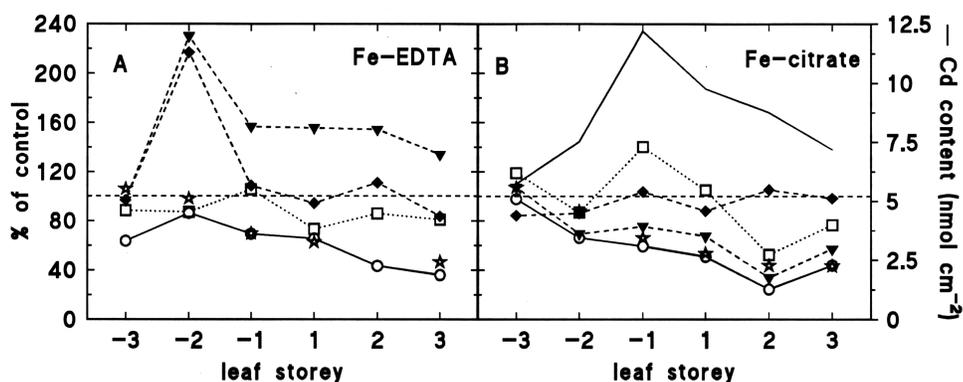


Fig. 1. Ion and Chl content of iron deficient poplar plants grown in Fe-EDTA prior to the treatment (A) and Cd treated ones grown in the presence of Fe-citrate (B). Values are expressed as the percentage of the corresponding control values. Control values were 24-26 $\mu\text{g Chl cm}^{-2}$, and 2 and 5-8 nmol cm^{-2} Fe and Mn, respectively. o - Fe, ▼ - Mn, (open square) - Ca, ◆ - K, ★ - Chl contents.

The amount of all Chl-proteins decreased parallel with the leaf iron content (Fig. 2). Correlation coefficient were 0.74-0.86 for photosystem (PS)II and 0.93-0.95 for all the other parameters. The lowering of the Chl *a/b* ratio with strengthening of the stress (Table 1) was due to a steeper decline in the amount of PSI (slopes: 1.110 and 1.283 in Fe deficient and Cd treated plants respectively) than in that of the light-harvesting complex (LHC)II (slopes: 0.918 and 1.068). It was accompanied by the relative decrease of the long wavelength fluorescence emission (not shown). PSII core turned out to be the most stable component under strong treatment. However, it was much more sensitive in Cd treated plants (slope: 0.844) than in Fe deficient ones (slope: 0.457). Nevertheless, PSII was more susceptible under mild stress than PSI or LHCII (Fig. 2A).

Similarly to the Chl content, the CO_2 fixation decreased parallel with the Fe content of leaves (Table 1). An increase in the non-photochemical quenching (NPQ) could be observed before any change in the F_v/F_m and the actual efficiency of PSII (Φ_{PSII}).

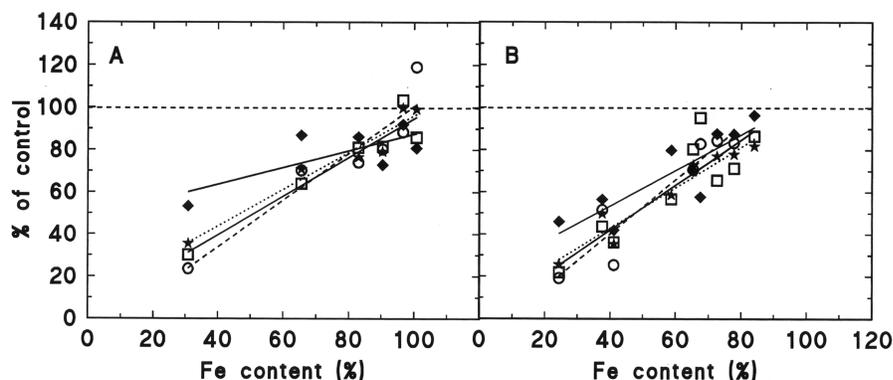


Fig. 2: Individual Chl and Chl-protein data ($\mu\text{g Chl cm}^{-2}$) from two independent experiments expressed in the percentage of the corresponding control values and plotted as the function of iron content (nmol cm^{-2}) also expressed in percentage of control. They were obtained from iron deficient (A) and Cd treated (B) leaves. \star - Chl, \circ - PSI, \blacklozenge - PSII, (opensquare) - LHCII.

It sharply rose under strong stress conditions, either Cd treatment or Fe deficiency, together with some decline in the F_v/F_m and Φ_{PSII} . An accompanying increase in VAZ/total carotenoids was also observed.

Table 1: Chl a/b ratio, carotenoids and fluorescence induction parameters measured in Cd treated and Fe deficient leaves grown during the treatment.

All values (mean values measured in the upper three leaves) are expressed as the percentage of the corresponding control value. Control Chl a/b values were around 3.55-3.70. F_v/F_m , Φ_{PSII} , and NPQ control values were 0.800-0.820, 0.55-0.60, and 0.45, respectively. Standard deviations were 2-15% of the measured values depending on the parameter and the treatment. VAZ/car – violaxanthin+ antheraxanthin+ zeaxanthin/ total carotenoid

<i>Sample</i>	Fe content	Chl a/b	CO_2 fixation	F_v/F_m	Φ_{PSII}	NPQ	VAZ/car
C – Fe def.	83.0	100.6	90.0	100.7	96.7	118.4	122.2
E – Cd tr.	37.6	93.3	35.6	97.1	89.2	142.2	126.2
C – Cd tr.	24.4	87.0	41.5	86.1	81.2	196.7	134.8
E – Fe def.	30.9	87.7	38.7	77.6	74.9	205.8	130.1

Discussion

The Chl content, i.e. the development of the photosynthetic apparatus, showed close correlation with the iron content of leaves. However, it was also connected with the inhibition of Mn accumulation in leaves under Cd treatment. The different susceptibility of Fe-EDTA- and Fe-citrate-grown plant to Cd treatment and Fe deficiency must be connected with the differential effect of chelators on the root adsorption, uptake and/or translocation of Fe and other ions in competition with heavy metals (Fodor *et al.* 1996).

Also the amount of all Chl-protein complexes declined in strong correlation with the iron content of leaves both in Fe deficient and Cd treated plants. The slope of the curves as the function of Fe content was the steepest for PSI and the slightest for PSII. The decreased accumulation of complexes may be connected with inhibition of their synthesis/stabilisation (Horváth *et al.* 1996, Sárvári *et al.* 1999) or due to their Cd induced degradation. Cd affected LHCII accumulation by reducing the steady-state level of Lhcb transcripts in bean (Tziveleka *et al.* 1999). Moreover, inhibition of its oligomerisation was shown in radish cotyledons due to the altered content of *trans*- Δ^3 -hexadecenoic acid (Krupa 1988). The stabilisation of PSI, which is particularly dependent on Fe availability, may be inhibited by Cd induced Fe-deficiency. Another possibility is its preferential degradation due to the inactivation of the protective enzymes by Cd (Gallego *et al.* 1996). PSII was found to be the most stable complex under strong stress. However, it was the most susceptible component under mild stress. In accordance, Franco *et al.* (1999) showed the modulation, stimulation and then inhibition of D1 protein turnover depending on the length of Cd stress. The steeper decline in the amount of PSII as the function of Fe content under Cd treatment than under Fe deficiency revealed that in the case of this complex Fe deficiency is not the only influencing factor, but another factor, perhaps Mn deficiency, also play a role in its accumulation.

However, compositional changes in thylakoids, i.e. the relative excess of PSII and decreased accumulation or degradation of LHCII, can also be explained by a regulatory mechanism working under excess irradiance, which may be evoked by Cd inhibition of photosynthesis. It was argued that stable dissipative PSII centres accumulate under photoinhibitory light (Anderson and Aro 1994). Sharp rise in non-photochemical quenching and increase in VAZ/total carotenoid content under strong or long Cd stress with moderately decreasing F_v/F_m and Φ_{PSII} values refer to such type of acclimation.

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