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ARTICLE

Effect of Cd on the iron re-supply-induced formation of chlorophyll-protein complexes in cucumber

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ABSTRACT Cd induced changes in the accumulation of chlorophyll-protein complexes were studied during the iron induced re-greening of iron deficient cucumber plants to see how the Cd-induced alterations develop during the thylakoid biogenesis, and whether the altered development of chlorophyll-protein complexes and the changed iron metabolism are connected. Treatment with 1 μM Cd resulted in strong decrease in iron content of leaves compared to the controls, but Cd could not be detected in the leaves. It was accompanied with strong retardation of chlorophyll synthesis and biogenesis of chlorophyll-protein complexes, particularly photosystem I, the amount of which even decreased at the later period of greening. During the re-greening, the actual efficiency of photosystem II and CO_2 fixation capacity recovered totally and partially, respectively. It was concluded that both retardation of the biogenesis of the complexes due to iron and chlorophyll deficiency and degradation processes affecting mainly photosystem I were involved in Cd induced changes in the pattern of chlorophyll-protein complexes.
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KEY WORDS

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Cd is a highly toxic divalent heavy metal, which is inhibitory for all living organisms. It damages numerous physiological processes in plants (van Assche and Clijsters 1990; Sanita di Toppi and Gabbrielli 1999), and photosynthesis proved to be particularly susceptible (Krupa and Baszynski 1995; Misliwa-Kurdziel et al. 2002). In addition to the inhibition of photosynthetic activity, the structure of the photosynthetic apparatus was also influenced. Leaf chlorosis (Misliwa-Kurdziel and Strzalka 2002), disorganisation (Krupa 1988) and reduced transcription of light-harvesting complex (LHC) II (Tziveleka et al. 1999) were observed.

Our previous studies revealed the alteration of the ratio of chlorophyll (Chl) containing complexes together with the lowering of Chl content and Chl *a/b* ratio in long-term Cd treated plants: the amount of all complexes reduced, photosystem (PS) I was the most affected while PSII core proved to be more resistant (Sárvári et al. 1999; Sárvári 2005). These symptoms of Cd treatment were similar to those of iron deficient plants (Abadia et al. 1989; Fodor et al. 1995; Timperio et al. 2007) in agreement with the fact that strong interaction has been observed between heavy metals and iron (Alcántara et al. 1994; Siedlecka and Krupa 1999). To see how these alterations develop during thylakoid biogenesis and whether the development of the Chl-protein pattern and the changed iron metabolism are connected or not, we studied the changes in the accumulation of Chl-protein complexes during the iron induced re-greening of iron deficient cucumber plants.

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Materials and Methods

Fe-deficient cucumber plants (*Cucumis sativus* L. cv. Budai korai) were grown hydroponically under standardised laboratory conditions in modified Hoagland solution of 1/4 strength (Fodor et al. 1998) but without iron supply for 19 days. 1 μM $Cd(NO_3)_2$ was added for a group of plants starting from the 20th day, and from the 21th day (up to 144h) re-greening of control and Cd treated plants was induced by the addition of 10 μM Fe-citrate without and with 1 μM $Cd(NO_3)_2$, respectively. Plants were grown and greened in growth chamber with 80-100 μmol m^{-2} s^{-1} light/dark periods of 14/10 h at 18/26°C and at about 70% humidity. Second leaves of plants grown and treated in three independent experiments were used.

Chl content was determined as in Porra et al. (1989). Chloroplasts, thylakoids and Chl-proteins were isolated according to Sárvári and Nyitrai (1994). A detergent mixture containing mainly glucosidic detergents (detergent/Chl = 10-15/1 w/w) were used for solubilisation: dodecyl sucrose (Calbiochem) : nonyl glucoside (SIGMA): lithium dodecyl sulphate (SIGMA) = 4.5:4.5:1. Chl-protein complexes were separated by Deriphat (Henkel) PAGE. Relative amounts of green bands were determined as the percentage of Chl applied on the gel by densitometry of the scanned gels using the Phoretix software (Phoretix International, UK). The amounts of bands identified by their polypeptide pattern as belonging to a given complex were summed up. The absolute amounts of specific complexes were calculated in μg Chl leaf $^{-1}$ by dividing the Chl content of a leaf among the complexes ac-

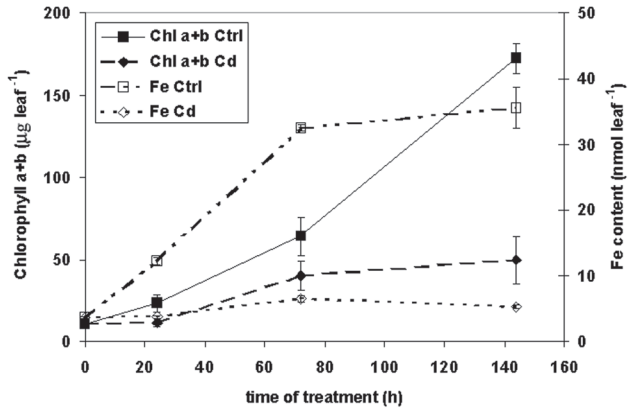

Figure 1. Changes in the iron (Fe) and Chl (Chl a+b) content of 2nd leaves of control (Ctrl) and Cd treated cucumber during the iron re-supply induced greening. 0 h means the start of the iron re-supply (10 µM Fe-citrate), and 1 µM Cd treatment started 24 h earlier.

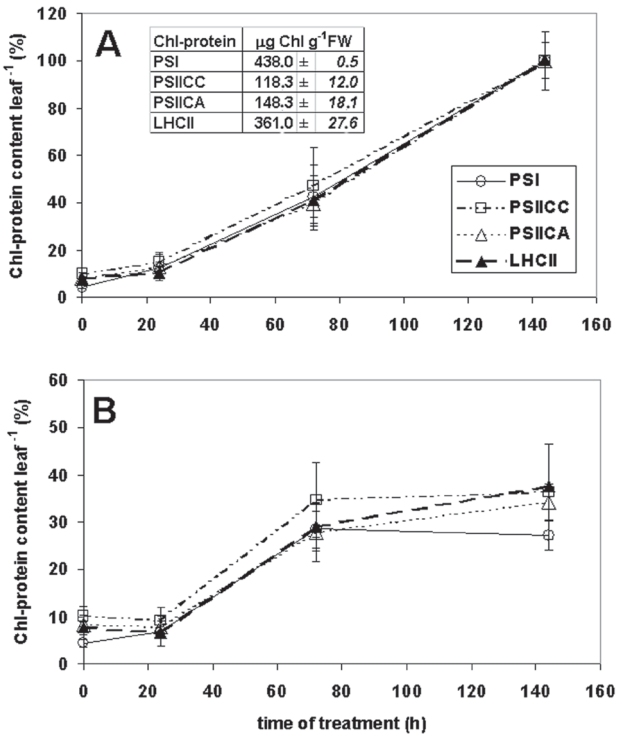

Figure 2. Changes in the Chl-protein content of 2nd leaves of control (A) and Cd treated (B) cucumber during the iron re-supply induced greening. 0 h means the start of the iron re-supply (10 µM Fe-citrate), and Cd treatment started 24 h earlier. Chl-protein content is given as the percentage of the values measured in control leaves after greening for 144 h. PSIICC – PSII core complex, PSIICA – PSII connecting antenna. Inset: Concentration of Chl-protein complexes in control leaves after 144 h. FW – fresh weight.

cording to their percentage proportions. Polypeptide patterns of the green bands were obtained on 10-18% gradient gels according to Laemmli (1970).

Fluorescence induction parameters were measured with a PAM fluorometer (Walz, Effeltrich, Germany). Light-induced CO_2 fixation was determined according to Láng et al. (1985).

Metal contents in dried plant tissue samples were determined by total reflection X-ray fluorescence spectrometry (TXRF) after wet digestion with HNO_3:H_2O = 1:1 (v/v) in a microwave-assisted digestion system (MDS 2100 CEM) (Varga et al. 1997). Analysis was carried out with an EXTRA IIA TXRF apparatus.

Results

Supply with iron induced rapid uptake of iron and greening of iron deficient cucumber plants. Iron content of control plants increased rapidly during the first 72 h of re-greening (Fig. 1) so that their iron concentration (213.8 ± 10.2 µg g^{-1} DW) approached that of the green leaves and then remained more or less constant. 1 µM Cd treatment extremely diminished the iron content of 2nd leaves, but it did not totally inhibited iron uptake and translocation. In contrast, Cd could not be detected in leaves treated with Cd at such a low concentration.

During the 144 h treatment, 2nd leaves of control and Cd treated plants were grown by only 50% and 20%, respectively. While the Chl content increased strongly and continuously in control leaves, Cd treated ones showed a 24 h lag period followed by a more vigorous increase in the next two days, then the accumulation of Chl slowed down, and only 28.7 ± 8.2% of the corresponding control value was reached by the end of the treatment (Fig. 1). The Chl synthesis was accompanied by changes in the Chl a/b ratio of iron deficient leaves (3.94 ± 0.44). In tendency, it elevated a little first then decreased to its final value, which was lower in Cd treated plants than in controls (3.21 ± 0.16 vs 3.56 ± 0.12).

Changes in the Chl a/b ratio of leaves were parallel to the reorganization of thylakoids. Compared to the green plants PSI and LHCII decreased more strongly than PSIICC in iron deficient leaves (PSIICC>LHCII>PSI). Both control and Cd treated leaves accumulated preferentially PSI in the first day of iron re-supply (Fig. 2). However, while PSIICC also accumulated and LHCII lagged in controls, reorganization of Chl from PSII and LHCII occurred in favour of PSI in Cd treated plants. During the later period of greening, all the complexes accumulated with nearly the same rate in control leaves. However, in Cd treated leaves a similar rate of accumulation was observed only in the next two days. Further on, the rate of accumulation of PSII and LHCII strongly decreased and the amount of PSI even reduced. Parallel to the reorganization of thylakoids, the low actual efficiency of PSII (0.390 ±

0.048) restored even in the presence of Cd (reaching 93.7 ± 3.8% of the control), while CO_2 fixation increased from 17.5 ± 6.0% in iron deficient leaves to 69.4 ± 19.0% of the control by the end of treatment.

Discussion

Re-greening of iron deficient cucumber plants in the presence of Cd gave the possibility to study the relationship between iron supply and Cd effects on the development of Chl-protein complexes. 1 μM Cd affected the leaf development in Fe-deficient cucumber plants similarly to that of 10 μM Cd in new leaves of plants supplied with iron (Sárvári et al. 1999), which also proved that iron deficient plants were more susceptible to Cd as it was shown in *Arabidopsis* by Rodecap et al. (1994). This was supposed to be related to the elevated uptake of Cd by the Fe-deficiency induced iron transporters (Hodoshima et al. 2007). However, Cd could not be detected in the 2nd leaves of plants re-greened with Cd at this low concentration, which referred to rather indirect effects of Cd on thylakoid development. It may be connected with strongly inhibited iron accumulation in these leaves.

The differences in the uptake and translocation of iron, both are competitively inhibited by Cd (Alcántara et al. 1994; Clemens 2006), may explain the extremely different rate of Chl accumulation in the control and Cd treated plants. Though Cd was shown to directly inhibit the synthesis of Chls (Padmaja et al. 1990), indirect inhibition of Chl biosynthesis by iron deficiency seems to be more probable in this case as Cd was not found in leaves. Iron deficiency is also known to influence Chl synthesis by controlling the activity of the Mg-protoporphyrin-IX monomethyl ester oxidative cyclase (Spiller et al. 1982).

In spite of the strongly inhibited Chl synthesis in Cd treated plants, thylakoid reorganization took place both in control and treated plants during the re-addition of iron. PSI reacted first to the increased iron supply. It refers to its higher sensitivity due to the extremely high iron content of the complex. However, stabilization of all complexes was retarded in Cd treated plants such as it was shown by Horváth et al. (1996). Both insufficient amount of iron and decreased Chl synthesis might have contributed to it, directly and indirectly, respectively. Decrease of the absolute amount of PSI at the final period of treatment, however, refers to secondary processes, which influence the stability of existing complexes. Iron deficiency induced inhibition of the energy usage (decreased CO_2 fixation) together with the fact that the actual efficiency of PSII was recovered, and in addition, the inhibition of protective enzymes (Benavides et al. 2005) led to the production of reactive oxygen species, which primarily damaged PSI. Therefore, the pattern of Chl-protein complexes developed in treated plants must be the result of all these processes, and both synthesis retardation due to iron and Chl deficiency and degradation processes could be involved.

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