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FINE STRUCTURAL CHANGES OF WHEAT PLASTIDS DURING CADMIUM INDUCED BLEACHING

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Introduction

Cadmium is one of the important environmental contaminants (Foy et al. 1978). It enters the plants either through the root, or directly from the air through the leaf surface. It is known that cadmium affects various metabolic activities in plants. Among others, it inhibits the chlorophyll synthesis and causes chlorosis of the leaves. It also inhibits the photosynthetic electron transport system, acting probably on the oxygen-evolving site of the photosystem II (Van Duivendijk-Matteoli and Desmet 1975, Baszyński et al. 1980). Preliminary investigations of the effect of cadmium on wheat plastids have shown that cadmium, when absorbed directly through the leaf surface, strongly affects greening of etiolated wheat leaves in light, and eventually produces their bleaching. Electron microscopic examinations have shown that the differentiation of etiochloroplasts is stopped at an early stage, and that — instead of normal grana thylakoids — abnormal membrane structures are formed, similar to those found in plastids of some chlorophyll deficient mutants.

Material and Methods

Wheat seedlings (*Triticum aestivum* cv. "super zlatna") were grown for 7 — 8 days either in complete darkness at 25 °C or at a photoperiod of 15 h light and 9 h darkness daily (2 fluorescent tubes 20 W, 4 500 K, giving 4000 lx at ground level). For experiments the second leaves were

detached from the seedlings and put into petri dishes on filter paper wetted either with a 0.1, 0.5 or 1 mmol solution of $CdCl_2$ in distilled water, or with distilled water alone. The leaves were held also on 1 mmol solution of $MnCl_2$, or on a solution containing both 1 mmol $CdCl_2$ and 1 mmol $MnCl_2$. The leaves were at first incubated for 4 hours in darkness and then exposed to light for 44 hours. Green leaves of seedlings grown in light were prepared and treated in a similar way.

For electron microscopic examinations small pieces of leaves were fixed in 1% glutaraldehyde in cacodylate buffer, postfixed in 1% OsO_4 and after dehydration embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I.

Chlorophyll was determined according to Arnon (1949, cited by Holden 1965). Plastids were isolated as described in a previous paper (Wrischer and Meglaj 1980). The photosynthetic activity (O_2 exchange) was measured with an O_2 electrode (Hansatech Ltd., Norfolk, England) as Hill reaction. The measurements (without addition of NH_4Cl) were performed as described earlier (Wrischer and Meglaj 1980).

Results

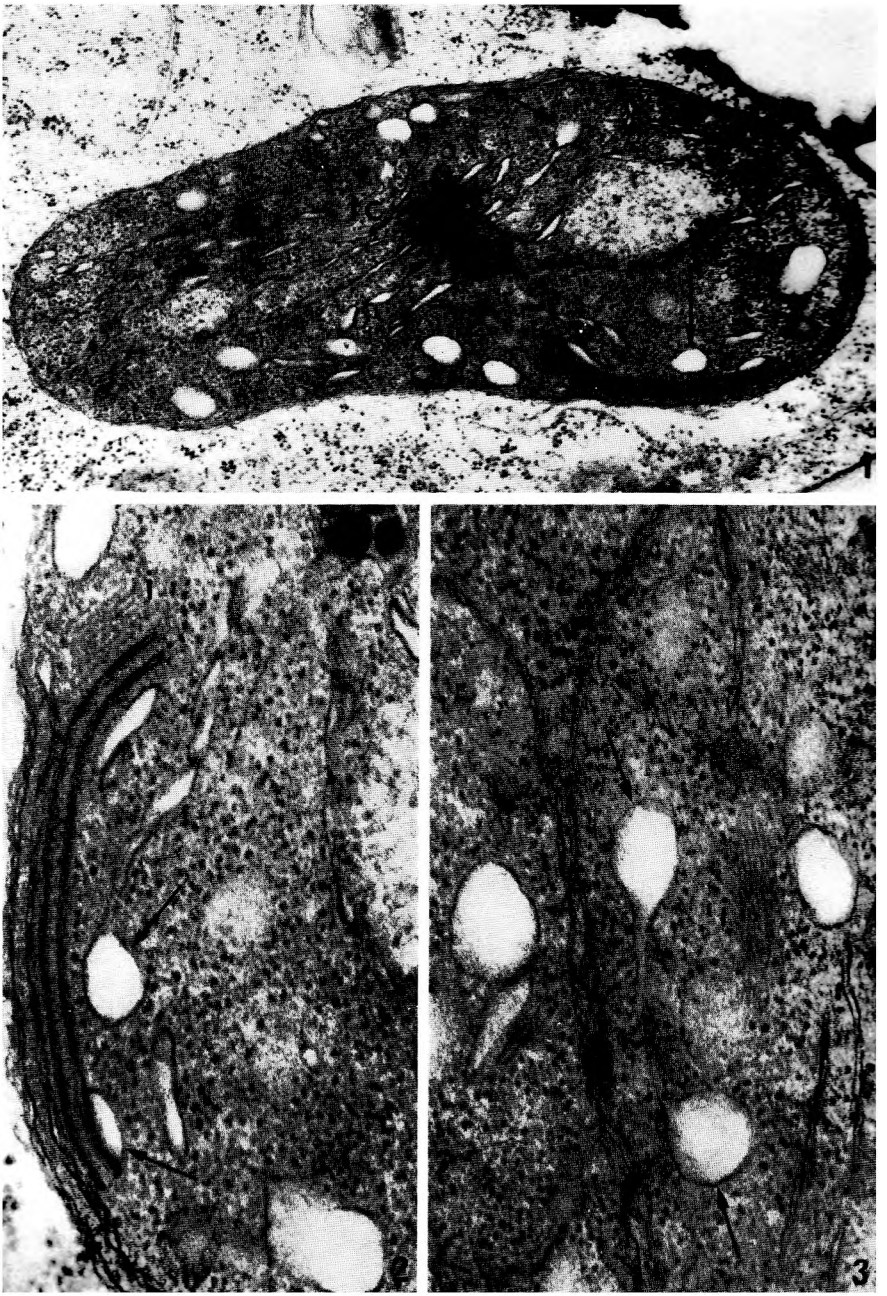
When etiolated leaves were held for 2 days in light on water, they became green and contained normal chloroplasts which had 0.87 mg/g fresh weight of chlorophyll, which was about 90% of the chlorophyll present in the leaves of intact seedlings (0.96 mg/g fr. wt.).

On the contrary, when etiolated leaves were held on a solution of $CdCl_2$, they remained yellow or became only slightly green. On a lower (0.1 mmol) concentration of cadmium the leaves succeeded to green somewhat and their chlorophyll content reached about 65% of that of control leaves. On higher concentrations of cadmium the inhibition of greening was much more pronounced. After 2 days on 1 mmol concentration of $CdCl_2$ the leaves were usually unevenly coloured. Only small portions of leaves succeeded in greening somewhat, while the others remained completely yellow, and after several days eventually became white. In these yellow leaf portions the transformation of etioplasts into chloroplasts was stopped at a rather early stage. Some of the plastids often contained

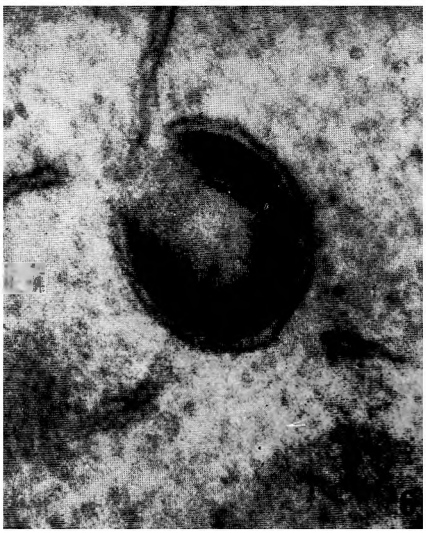
Figs. 1—7.

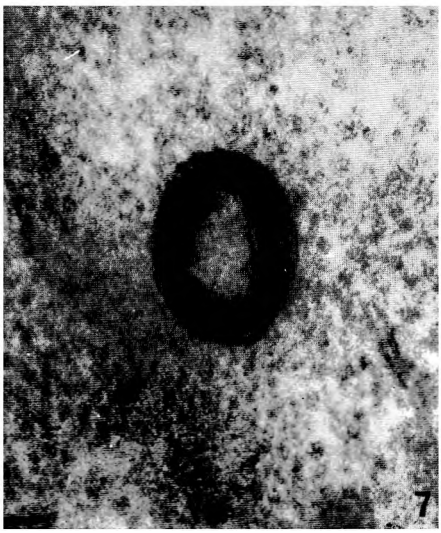
Plastids from yellow portions of etiolated wheat leaves held for 2 days in light on 1 mmol solution of $CdCl_2$.

Fig. 1. Plastid with a tubular complex (tc) and prothylakoids dilated at their ends (arrows). 31,000 : 1.
Fig. 2. Detail of Fig. 1. with dilated prothylakoids (arrows) and a fibrillar inclusion in the stroma (i). 72,000 : 1.
Fig. 3. Part of a plastid with dilated prothylakoids (arrows) and a fibrillar inclusion in the stroma (i). 60,000 : 1.
Fig. 4. Plastid with individual prothylakoids, a cup shaped stack of thylakoids (t), and a fibrillar inclusion in the stroma (i). 38,000 : 1.
Fig. 5. Detail of Fig. 4. with a cup shaped stack of appressed thylakoids. 120,000 : 1.
Fig. 6. Part of a plastid with a stack of bent thylakoids. 120,000 : 1.
Fig. 7. Part of a plastid with the cup shaped thylakoid stack cut tangentially. 120,000 : 1.



Figs. 1—3.





Figs. 4—7.

tubular complexes (remnants of prolamellar bodies) and only few prothylakoids which were often dilated at their ends (Figs. 1 — 3). In other plastids new cup shaped structures were formed. They consisted of groups of appressed and bent thylakoids (Figs. 4 — 7). Although the thylakoid system was strongly affected by cadmium, the plastid stroma appeared normal. It was full of ribosomes (Figs. 2, 3, 5, 7), and fibrillar protein inclusions, which are known to appear occasionally in the stroma of wheat chloroplasts (Gunning et al. 1968), were quite often present (Figs. 2—4).

The chlorophyll content of yellow leaves reached only 38% (0.37 mg/g fr. wt.) of that of the control ones. Although the Hill reaction of these plastids was low (1.20 μmol O_2/g fr. wt./h) in comparison with the control (2.76 μmol O_2/g fr. wt./h), their photosynthetic efficiency (when calculated to the chlorophyll present in them) was even higher than in the control.

The inhibition of greening caused by cadmium could be reversed only when the leaves were held for a short time (3 — 4 hours) on cadmium and were then transferred to water. A longer treatment with cadmium caused irreversible damage of the plastids. The addition of $MnCl_2$ (1 mmol) only partly suppressed the effect of cadmium: after 2 days in light the leaves contained chloroplasts with very small grana and a chlorophyll content which reached about 45% of that of the control leaves.

When green leaves (of seedlings grown in light) were held on cadmium (1 mmol), there were no significant changes in the fine structure of their chloroplasts, although both the chlorophyll content and the Hill reaction were lower than in the control. After two days on 1 mmol solution of $CdCl_2$ the chlorophyll content dropped to about 67% (0.94 mg/g fr. wt.) of the control (1.39 mg/g fr. wt.). The Hill reaction was somewhat less inhibited and reached about 55% (3 μmol O_2/g fr. wt./h) of that in untreated chloroplasts (5.4 μmol O_2/g fr. wt./h).

Discussion

Cadmium is a very efficient inhibitor of plastid differentiation. The transformation of etioplasts into chloroplasts in light is stopped very early. Moreover, abnormal membrane structures are formed *de novo*. Similar appressed and bent thylakoid stacks have been described e. g. in yellow portions of temperature bleached "golden leaf" mutant of maize (Orsenigo and Marziani 1971), in chlorophyll deficient mutants of barley (Nielsen et al. 1979), and in yellow leaves — grown in strong sunlight — of some "aurea" varieties (Wrischer et al. 1976). All these mutant plants are defective in chlorophyll synthesis. As has been shown in this paper, cadmium preferentially inhibits chlorophyll synthesis. In both etiolated and green wheat leaves the percentage of inhibition of chlorophyll synthesis is always higher than the percentage of inhibition of the Hill reaction. The same effect has been observed also in leaves of plants grown on a medium containing cadmium (Baszyński et al. 1980). This preferential inhibition of chlorophyll synthesis is also the cause of the high photosynthetic efficiency of cadmium treated etiochloroplasts, and at the same time it indicates that the etioplast-chloroplast transformation is stopped at a very early stage (Plesničar and Bendall 1973).

The addition of manganese only partly suppresses the inhibitory effect of cadmium on the plastid differentiation. This fact is in good accordance with the results of other investigators, namely that manganese only partly reverses the inhibition of photosynthesis and chlorophyll synthesis in isolated chloroplasts as well as in green plants treated with cadmium (Van Duivendijk-Matteoli and Desmet 1975, Baszyński et al. 1980).

Summary

Cadmium, when applied directly through the leaf surface, affects the greening of etiolated leaves in light and eventually produces their bleaching. After 2 days on 1 mmol solution of $CdCl_2$, in yellow leaf portions the transformation of etioplasts into chloroplasts is strongly inhibited. The plastids contain tubular complexes (remnants of prolamellar bodies), some dilated prothylakoids, and stacks of appressed and bent cup shaped thylakoids. The chlorophyll synthesis and the photosynthetic activity (Hill reaction) of these plastids are very low, although the photosynthetic efficiency is higher than in the control. The changes described are discussed in connection with similar changes in some chlorophyll deficient mutants.

Athough the fine structure of chloroplasts of green leaves treated with cadmium is not considerably altered, the concentration of chlorophyll and the photosynthetic activity (Hill reaction) are much lower than in the control leaves.

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SAŽETAK

ULTRASTRUKTURNE PROMJENE U PLASTIDIMA LISTOVA PŠENICE IZAZVANE KADMIJEM

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Ako se etiolirani listovi pšenice izlože djelovanju kadmija, njihovo je ozelenjavanje snažno zakočeno. Listovi ostanu pretežno žuti, a nakon nekoliko dana posve izblijede. U žutim dijelovima listova, koji su dva dana bili na površini otopine $CdCl_2$ (1 mmol), prijetvorba etioplasta u kloroplaste je zakočena. Plastidi sadržavaju tubularne komplekse (ostatke prolamelarnih tjelešaca), malobrojne dilatirane protilakoide i svežnjeve čaškasto svinutih tilakoida. Sinteza klorofila i fotosintetska aktivnost (Hillova reakcija) plastida vrlo su niske, dok je fotosintetska efikasnost viša nego u kontroli. Opisane promjene uspoređene su sa sličnim promjenama u nekim mutantama deficijentnim u klorofilu.

Ultrastruktura kloroplasta zelenih listova tretiranih kadmijem nije bitnije izmijenjena, iako je i koncetracija klorofila i fotosintetska aktivnost znatno niža od one kontrolnih listova.

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