Variation in Photosynthetic Pigments and Plastoquinone Contents in Sugar Beet Chloroplasts with Changes in Leaf Copper Content

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ABSTRACT

The changes in the contents of chlorophyll (Chl) a, Chl b, carotenoid, and plastoquinone in sugar beet (Beta vulgaris) chloroplasts were investigated during Cu deficiency and resupply. With the onset of Cu deficiency, extrachloroplastic Cu decreased more rapidly than chloroplast Cu, which eventually accounted for all of the Cu present in the leaf. The ultrastructure of Cu-deficient chloroplasts did not differ from the control except in very young, severely deficient leaves. During Cu depletion and resupply, Chl a, Chl b, carotenoid, and plastoquinone contents changed concomitantly. Because of the concurrent changes in the contents of terpenoid-containing pigments and plastoquinone with Cu depletion and resupply, we suggest that Cu might be involved in the regulation of the early steps of terpenoid biosynthesis prior to the formation of geranylgeranyl-pyrophosphate.

Copper is known to be an essential micronutrient in both algae and higher plants (3, 21). Its deficiency in mineral nutrition results in a decrease in photosynthetic activities (5, 14). Cu deficiency was found to reduce PSI electron transport and to diminish the plastocyanin content of chloroplasts (4, 15). Thus, most investigators believe that Cu deficiency effects on photosynthetic electron transport are mediated via plastocyanin (3, 4). Cu deficiency has other characteristic symptoms which include necrosis and chlorosis (9, 11). The chlorosis has been characterized histologically as the discontinuous distribution of Chl within the chloroplasts (9). Vesk et al. (20) found no ultrastructural alteration between the Cu-deficient and control chloroplasts. Baszynski et al. (4) noted some depletion of chloroplast membranes with Cu deficiency. Investigating the effect of Cu deficiency in two different genera, they also found that Cu deficiency decreased the amounts of photosynthetic pigments and lipoquinones to varying extents and concluded that Cu deficiency inhibited the synthesis of these individual chloroplast components differently. However, this conclusion was based on observations made at a static stage of the fully developed deficiency.

In the present work, we followed the changes with time in the contents of Chl, carotenoid, and plastoquinone of sugar beet chloroplasts during the development of Cu deficiency. In agreement with Baszynski et al. (4), we found large decreases in the amounts of photosynthetic pigments and lipoquinones; however, we also found that the time courses of the changes in photosynthetic pigments and plastoquinone were highly correlated. From this we concluded that Cu deficiency interfered with one of the early step(s) of terpenoid biosynthesis.

MATERIALS AND METHODS

Sugarbeet (Beta vulgaris cv F58-554H1) was grown hydroponically in growth chambers at 25°C and illuminated at 40,000 lux for 16 h/day. The plants were cultured for 2 weeks following planting in vermiculite with half-Hoagland solution (18). After 2 weeks, the plants were transferred to half-Hoagland solution without Cu and grown for 2 to 3 weeks.

Intact chloroplasts were isolated essentially as described by Thorne et al. (19). About 20 g of leaves were ground in a Waring Blender for 4 to 5 s in a medium containing 0.4 mm sorbitol, 10 mm NaCl, 5 mm MgCl2, 1 mm MnCl2, 2 mm EDTA, 2 mm ascorbate, 0.4% BSA, and 50 mm Mes (pH 6.5); for the leaf homogenization, this basal isolation medium was supplemented with 1% PVP and 10 mm ascorbate. The brei was filtered through four layers of Miracloth and the chloroplasts were sedimented by centrifugation for 45 s at 2000g. The chloroplasts were washed once in basal isolation medium and resuspended in a medium containing 0.4 mm sorbitol, 10 mm NaCl, 5 mm MgCl2, 1 mm MnCl2, 2 mm EDTA, 0.4% BSA, and 50 mm Hepes (pH 7.5), to give a Chl concentration of 1.0 to 1.5 mg/ml. The content of Cu in the leaf and chloroplast samples was determined using an atomic absorption spectrophotometer with flame atomization (Varian model AA6). The samples for atomic absorption analysis were dried and digested in a mixture of 85% HNO3 and 15% HClO4.

Chl content was determined in 80% acetone extract according to the method of Arnon (2). Carotenoid content was estimated on the basis of the A at 480 nm using an absorption coefficient of εmax = 2000 (9).

Plastoquinone (PQ) content was determined from a lipid extract of the chloroplasts according to Redfearn and Fried (16). The amount of oxidized and reduced PQ was measured by adding to the sample a few crystals of K3Fe(CN)6 or NaBH4, respectively.

The pigment-protein complexes were separated by LiDS-PAGE. Except for minor modifications, the procedures were identical to those of Delepelaire and Chua (6). Prior to the solubilization of the pigment-protein complexes, the chloroplasts were washed with a buffer containing 40 mm sucrose, 10 mm NaCl, 5 mm MgCl2, and 30 mm Tricine (pH 7.8).

For electron microscopy, leaf samples were fixed, dehydrated, embedded, sectioned, and stained by conventional methods (13). O2 evolution and consumption were measured using a Clark-type electrode as described previously (7).

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3 Abbreviations: PQ, plastoquinone; LiDS, lithium dodecyl sulfate.
Fig. 1. Variation in the Cu contents of leaves (●, ○) and chloroplasts (▲, △) of plants during development with (●, ▲) and without (○, △) Cu. (Cu was omitted from the culture solution at time zero on the abscissa).

Fig. 2. Changes in total Chl content and Chl a/b ratios in leaves of plants with and without supplied Cu during plant development. A, Total Chl content of control (●) and Cu-deficient leaves (○); B, Chl a/b ratio in control (●) and Cu-deficient chloroplasts (○).

RESULTS

The changes in the Cu contents of leaves and chloroplasts of both control and Cu-deficient plants are shown in Figure 1. During development, control plants showed a 30% decrease in leaf Cu content, which we attribute to dilution during the rapid phase of vegetative growth. The removal of Cu from the culture medium resulted in a large, rapid decrease in leaf Cu content, with more than 80% of the total Cu being lost over the 2.5-week period. Chloroplast Cu content (calculated on a per area basis) showed similar changes over time with those of leaf Cu content. In the early stages of deficiency, extrachloroplastic Cu decreased more than chloroplast Cu and, after 1 week, only chloroplast Cu was present in the leaves.

The decrease of Cu in the leaves and/or chloroplasts was accompanied by decreases in pigment content. The Chl content of Cu-deficient chloroplasts decreased steadily after about 1 week; in control plants, Chl increased (Fig. 2A). After 2.5 weeks without Cu, more than 50% of the total Chl content per leaf area was lost. The Chl a/b ratio was not changed by Cu deficiency even in severely deficient chloroplasts (Fig. 2B).

Both control and Cu-deficient chloroplasts had essentially the same pigment protein composition (Fig. 3). During electrophoresis of Cu-deficient chloroplasts, the amount of free pigment was increased with the length of time that the gel was run. We therefore used a shorter running time which improved the situation somewhat but there was still a greater proportion of free pigment than in the control. The increase in free pigment was mostly at the expense of LHCP3 (terminology according to Anderson et al. [1]). This suggests that the light-harvesting pigments in the Cu-deficient plants were less stable than in the control.

The total carotenoid content of the leaves was decreased by 50% after 2.5 weeks of Cu deficiency (Fig. 4). The fact that Cu deficiency changed both Chl and carotenoids to a similar extent suggests that Cu deficiency influenced either the amount of thylakoids per leaf area or the synthesis of pigments.

Electron micrographs of Cu-deficient and control chloroplasts are shown in Figure 5. It is clear that Cu deficiency had little or no effect on chloroplast ultrastructure: only in very young leaves of extremely deficient plants was there any disorganization (Fig. 5C). These very small leaves accounted for no more than 5% of the total leaf mass.

Plastoquinone content showed very similar changes to those of the Chl and carotenoids (Fig. 6). After 2.5 weeks of Cu deficiency, the PQ content per unit area had diminished by 50%.
CHANGES IN PIGMENTS AND PLASTOQUINONE WITH LEAF Cu

Fig. 5. Electron micrographs of chloroplasts in control and Cu-deficient leaves. A, Control leaf (× 28,000); B, Cu-deficient leaf (× 49,000); C, very young, severely Cu-deficient leaf (× 30,000).

Although the total amount of PQ on a leaf area basis was decreased strongly after 1 week, the Chl/PQ ratio was not different in the control and Cu-deficient chloroplasts even after 3.5 weeks of Cu deficiency (Fig. 6).

During the first 72 h of resupply, Chl and carotenoid contents did not change significantly (Fig. 7). They returned to control levels after 7 d of resupply. Photosynthetic electron transport calculated on a per area basis began to increase after 24 h of resupply, and was recovered completely after 96 h (Fig. 8). The elevated activity on a per Chl basis clearly indicates that delayed membrane synthesis was not responsible for the decreased contents of pigments and PQ.

**DISCUSSION**

It has been shown previously that Cu deficiency decreased the amount of both photosynthetic pigments and prenol lipids in the

**Fig. 6.** Changes in plastoquinone content in leaves of plants with and without supplied Cu. A, Total plastoquinone contents of control (●) and Cu-deficient chloroplasts (○); B, Chl/plastoquinone ratio in controls (●) and Cu-deficient chloroplasts (○).

**Fig. 7.** Changes in Chl (●) and carotenoid (○) content of Cu-deficient leaves after resupply of Cu to the culture medium. Data are given as percentage of the control.

**Fig. 8.** Change in photosynthetic electron transport activities after resupply of Cu to the culture medium. Data expressed as percentage of the control (plants grown in half-Hoagland solution throughout the period of experiments). The rate of the control chloroplasts was: A, H₂O →FeCy (195 μmol O₂/mg Chl·h); B, H₂O →PBQ (228 μmol O₂/mg Chl·h); C, DCIP →MV (300 μmol O₂/mg Chl·h). (●●●), Activity calculated on Chl basis; (○○○), activity calculated in leaf area basis.

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chloroplasts (4). The results of the present work show clearly that, during Cu depletion and resupply, the amounts of Chl, carotenoids, and plastocyanin changed concomitantly. One interpretation of these data is that Cu might be required for the formation of thylakoids. There was no indication from the present work that Cu deficiency reduced the amount of thylakoid material per chloroplast and there was no effect on chloroplast ultrastructure except in very young, severely deficient leaves. Vesk et al. (20) similarly found no evidence of loss of thylakoid material with Cu deficiency. Furthermore, we found that the thylakoid protein content (per Chl) of deficient chloroplasts was nearly double that of the control; the protein/Chl ratio was 25 and 11 in the deficient and control chloroplasts, respectively. Thus, it seems unlikely that the effects of Cu depletion and resupply were mediated via changes in the amount of thylakoids.

An alternative explanation for the concomitant changes in photosynthetic pigments and plastocyanin contents with Cu depletion and resupply, is that Cu may be involved in some way in the biosynthesis of these compounds. It is generally accepted that there is a common path in the biosynthesis of Chl, carotenoids, and quinones at the point of terpenoid biosynthesis (8). The synthesis of the C30 phytol and C40 carotenes diverge from terpenoid synthesis after the formation of geranyl-geranyl pyrophosphate and the C40 side chain of plastocyanin is synthesized via solanesyl-45-pyrophosphate (8, 12). Thus, the concomitant changes in pigment and plastocyanin content suggests that Cu could affect terpenoid biosynthesis prior to the formation of geranyl-geranyl pyrophosphate.

Enzymes which are known to be involved in the early steps of terpenoid biosynthesis are not known to require Cu (15). This suggests that a direct involvement of Cu at this stage of terpenoid biosynthesis is unlikely; however, it should be pointed out that this pathway is largely based on animal rather than plant enzyme systems (15).

Electron transport activity began to recover only 24 h after Cu was resupplied and completely recovered after 4 d. The chloroplast contents of photosynthetic pigments and plastocyanin, however, did not begin to increase until after 72 h and did not reach control levels until 7 d had elapsed. This suggests that the effect of Cu on pigment and plastocyanin synthesis may have been indirect: if, for example, Cu is required for the synthesis of enzymes involved in terpenoid biosynthesis, one might anticipate a delay before the photosynthetic pigments and plastocyanin were synthesized. Thylakoid content, however, was presumably at normal levels after only 4 d because electron transport activity on a per area basis was fully recovered by that time.

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LITERATURE CITED