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Iron Nutrition-Mediated Chloroplast Development

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ABSTRACT

Membrane development in chloroplasts was explored by resupplying iron to iron-deficient sugar beet (*Beta vulgaris* L. cv F58-554H1) and monitoring changes in lamellar components during regreening. The synthesis of chlorophyll *a*, chlorophyll *b*, and Q, the first stable electron acceptor of photosystem II, exhibited a lag phase during the first 24 to 48 hours of resupply. In contrast, the per area amounts of P_{700} and cytochrome *f* increased linearly over the first 48 hours. During the early regreening period, the Q to P_{700} ratio was 2.6 and decreased to 0.7 after 96 hours of regreening. The rate of photosynthesis (net CO_2 uptake) per chlorophyll increased during the first 48 hours of resupply, then by 96 hours decreased to values typical of control plants. The results suggest that there was preferential synthesis of the measured photosystem I components during the first 24 to 48 hours, while from 48 to 96 hours there was rapid synthesis of all components. The iron nutrition-mediated chloroplast development system provides a useful experimental approach for studying biomembrane synthesis and structural-functional relations of the photosynthetic apparatus.

A number of experimental systems have been utilized to study the development of chloroplasts. These include the study of the etioplast to chloroplast transformations mediated by growth under various light regimes (23), studies involving mutants (12, 15, 29, 30), and work with various sections of leaves (18, 21).

The chloroplast development system presented here uses a different approach, which involves the resupply of Fe to Fe-deficient sugar beet plants. Earlier research showed that when a healthy sugar beet plant is grown in an Fe-deficient nutrient solution for 6 to 8 d, the light-harvesting and electron transport system components are preferentially decreased (24, 25, 28). When Fe is resupplied, thylakoids are synthesized and chlorosis is reversed (19, 27). Thus, resupplying Fe to Fe-deficient plants permits the study of *de novo* synthesis of chloroplast lamellae over a period of 96 h.

The objective of the present investigation was to characterize the changes in certain PSI and PSII components during the regreening period and to correlate these changes with photosynthesis *in vivo*. To this end, we monitored changes in Chl *a*, Chl *b*, P_{700}, Cyt *f*, Q, and the rate of photosynthetic CO_2 uptake *in vivo*.

MATERIALS AND METHODS

Plant Culture. Sugar beets (*Beta vulgaris* L. cv F58-554H1) were cultured as described previously (28). After 6 to 8 d without Fe, uniformly chlorotic plants were transferred to pots containing a complete nutrient solution. Rapidly expanding leaves (each 125–175 cm^2) were harvested at four times (within 2 h of the start of the photoperiod), once prior to transfer to Fe-containing nutrient solution, and at 24, 48, and 96 h after transfer. Leaf samples were immediately placed in plastic bags and stored at 4°C until assayed (usually within 2 h).

Experiment 1. Cyt *f*, P_{700}, Chl *a*, and Chl *b* were monitored during Fe resupply. Five to six replicates were made at each time point (Table I).

Experiment 2. Q, P_{700}, Chl *a*, and Chl *b* were monitored during Fe resupply. Each measurement represents a pooled sample of 5 to 13 leaves (Table I).

Cyt *f* and P_{700} Determination. Cyt *f* and P_{700} were extracted according to the procedure used by Spiller and Terry (24) for Cyt *f*, with the following modification: after washing and resuspension, the chloroplast pellet was solubilized in 1% (v/v) Triton-X 100 (in 50 mM Tricine-KOH and 5 mM $MgCl_2$) to give a final concentration of 90 to 370 μM Chl. The samples were allowed to sit at room temperature for 30 min before centrifuging at 12,500*g* for 1 to 2 min. A small aliquot for P_{700} determination was removed, diluted, and enough 0.5 M Na-ascorbate added to give a final concentration of 30 mM.The remaining supernatant was used for Cyt *f* determination. Cyt *f* and P_{700} were measured as described (24).

Determination of the Primary Electron Acceptor Q. Leaf tissue (midrib removed) was torn into small pieces and homogenized in a Waring Blendor for 5 s in an extracting solution consisting of 0.4 M sorbitol, 20 mM Tricine, 10 mM $MgCl_2$, 5 mM EDTA, 2 mM Na-ascorbate; pH adjusted to 7.8 at 2°C with KOH. The leaf brei was filtered through six layers of fine nylon mesh and centrifuged at 1200*g* and 2°C for 2 min. The pellet was resuspended in a buffer solution consisting of 50 mM Tricine, 10 mM NaCl, 5 mM $MgCl_2$, and the pH adjusted to 7.8 at 2°C with KOH; it was then centrifuged at 18,000*g* and 2°C for 10 min. To insure complete removal of Na-ascorbate, the wash was repeated. The washed pellet was resuspended in the buffer solution with the aid of a ground glass homogenizer, and a 0.1-ml aliquot was removed for Chl determination. The measurement of Q was by the procedure of Melis and Brown (16).

Chl Determination. Chl *a* and Chl *b* content were determined in 80% acetone (3).

Photosynthesis. Photosynthetic activity was determined by open flow gas exchange analysis (26). The rate of photosynthetic CO_2 uptake was measured at 30°C and saturating levels of CO_2 (1,000 μl l^{-1} CO_2) and irradiance.

RESULTS

When Fe was resupplied to Fe-deficient plants, the per area amount of Cyt *f*, which tripled from 39 to 119 pmol cm^{-2} over the 96-h period (Table I), increased linearly for the first 48 h and then appeared to increase at a slower rate over the next 48 h (Fig. 1, experiment 1). The per area amount of P_{700} increased linearly throughout the 96 h period from an initial value of 34 to 154 pmol cm^{-2} (Fig. 1; Table I, experiment 1). Unlike P_{700} and Cyt *f*, Chl *a* and Chl *b* exhibited a lag phase of 24 to 48 h before rapid synthesis occurred (Fig. 1, experiments 1 and 2). The ratio of Chl *a* to Chl *b*, which was already elevated in Fe-deficient plants, increased over the first 24 h before it decreased to control levels (Table II), presumably due to the lack of Chl *b* synthesis during the first 24 h after Fe resupply (Fig. 1). The ratio of Chl (*a*+*b*) to P_{700}, which

Table I. *Per Area Amounts of Cyt f, P_{700}, Q, and Chl a + b after Resupply of Iron to Iron-Deficient Sugar Beet Plants*

Parameter	Exp No.	Time (h)				
		0	24	48	96	Control
Cyt *f* (pmol cm^{-2})	1	39 ± 3	63 ± 9	89 ± 17	119 ± 10	156 ± 37
P_{700} (pmol cm^{-2})	1	34 ± 3	58 ± 6	79 ± 9	154 ± 13	161 ± 31
	2		25	63	157	186
Q (pmol cm^{-2})	2	50	65	58	114	154
Chl *a* + *b* (nmol cm^{-2})	1	13 ± 1	16 ± 2	21 ± 3	44 ± 4	49 ± 8[a]
	2	8	9	19	45	47
Sample size	1	5	5	5	6	25[a]
No. leaves/sample[b]	2	6	13	9	6	5

[a] This represents the value for control plants for which P_{700} was determined; control plants for which Cyt *f* was determined had an average Chl concentration of 59 ± 7 nmol cm^{-2} (n = 17).

[b] The values for experiment 2 represent single determinations on the pooled leaf samples.

Table II. *Change in Chl a/b after Resupply of Iron to Iron-Deficient Sugar Beet Plants*

	Time (h)				
	0	24	48	96	Control
Chl *a/b*	5.31 ± 0.70	7.53 ± 0.71	5.71 ± 0.33	4.19 ± 0.13	4.19 ± 0.18
Sample size	65	33	31	26	31

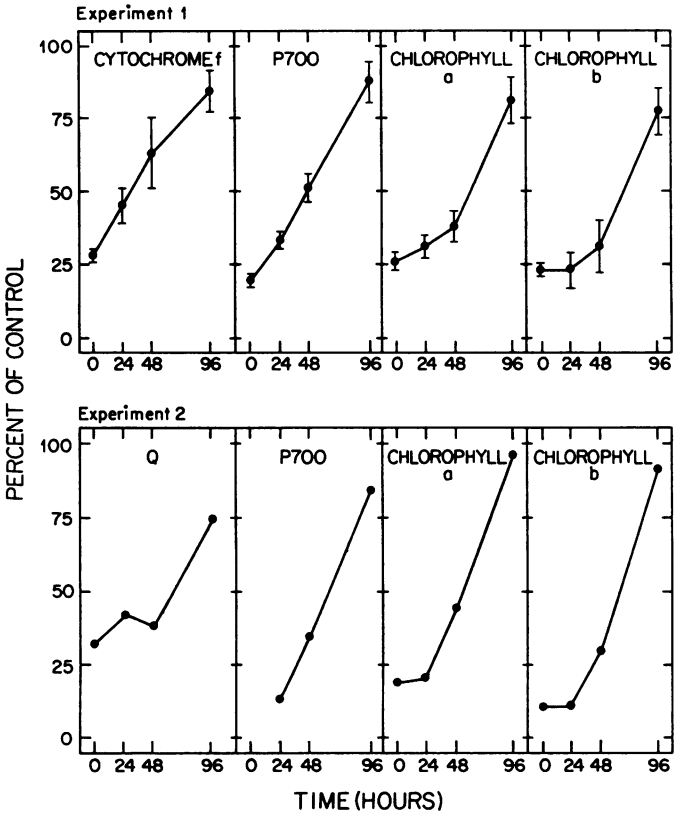

FIG. 1. Changes in per area amounts as a percent of control of Cyt *f*, P_{700}, Q, Chl *a*, and Chl *b* after resupply of Fe to Fe-deficient sugar beet plants. Experiment 1, Fe was withheld 6 days. Experiment 2, Fe was withheld 7 d; each point represents pooled sample of 5 to 13 leaves. Control based on a Chl concentration of 55 nmol/cm^2.

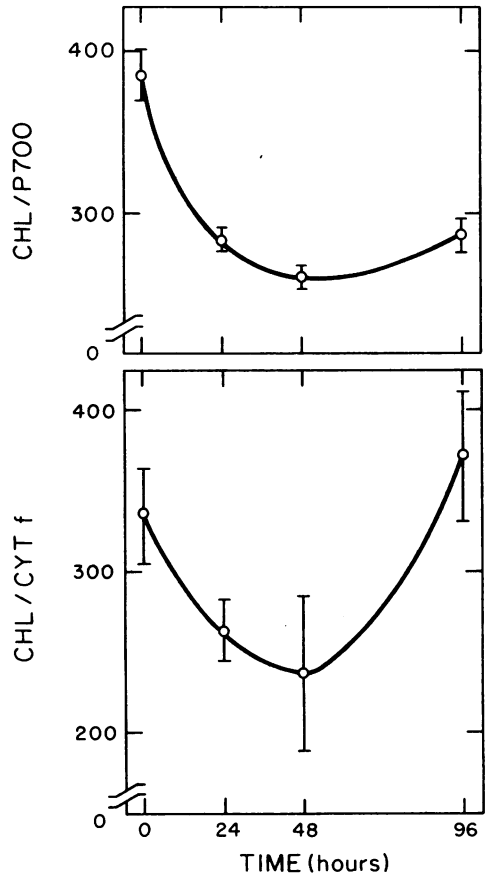

FIG. 2. Changes in Chl/P_{700} and Chl/Cyt *f* during redevelopment of sugar beet chloroplasts after resupply of Fe to Fe-deficient plants. Data from experiment 1.

was elevated in Fe-deficient plants, decreased to a minimum at 48 h, and increased to control values by 96 h (Fig. 2). The Chl to Cyt *f* ratio, which was unchanged by Fe deficiency, also decreased to a minimum at 48 h and by 96 h had returned to the control level (Fig. 2).

In one resupply experiment (experiment 2), the PSII component Q was measured in addition to P_{700}, Chl *a*, and Chl *b* (Fig. 1, experiment 2). Q exhibited a lag phase of 48 h and increased from 50 to 114 pmol cm^{-2} after 96 h, whereas P_{700} increased linearly

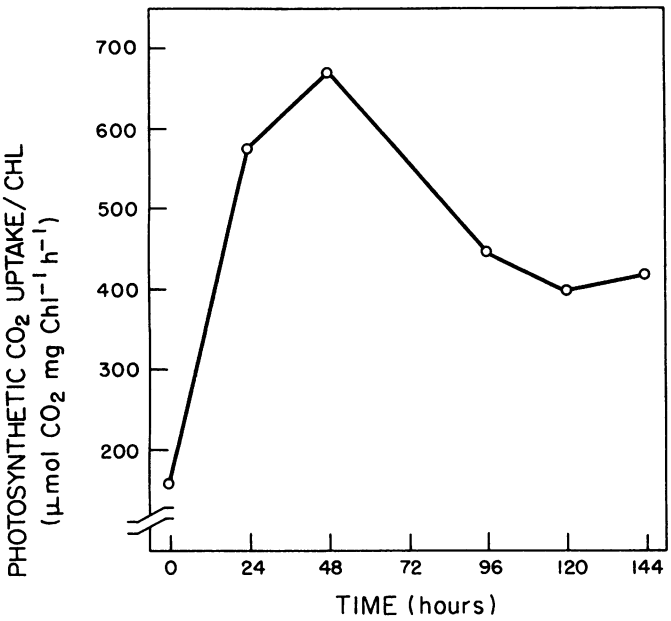

FIG. 3. Changes in photosynthetic CO_2 uptake per Chl after resupply of Fe to Fe-deficient sugar beet plants. Photosynthesis measured as described in "Materials and Methods."

from 25 to 157 pmol cm^2 over the 24 to 96 h period (Fig. 1; Table I). Thus, Fe deficiency reduced P_{700} more than Q, and Fe resupply resulted in a greater increase in P_{700} than in Q. Experiment 2 differed from experiment 1 in that plants in experiment 2 were initially more chlorotic than those of experiment 1 (Table I).

Light-saturated net CO_2 exchange rates of single attached leaves increased from 157 μmol CO_2 mg^{-1} Chl h^{-1} in Fe-deficient plants to 667 μmol CO_2 mg^{-1} Chl h^{-1} after 48 h of Fe resupply (Fig. 3). By 96 h, the photosynthetic rate returned to control levels (approximately 400 μmol CO_2 mg^{-1} Chl h^{-1}).

DISCUSSION

A major difference between the Fe nutrition-mediated chloroplast development system described here and the light-activated etioplast to chloroplast transformation is that chloroplasts in Fe-deficient leaves contain no prolamellar body. Hence, the Fe nutrition-mediated system involves *de novo* synthesis of the thylakoids without any apparent prolamellar body stage (19). When thylakoid development is initiated by Fe resupply in Fe-deficient plants, it is accompanied by a preferential synthesis of P_{700} compared to the bulk Chl as reflected by the decrease in Chl/P_{700} ratio. This is similar to the etioplast to chloroplast transformation, in which the Chl to P_{700} ratios also rapidly decrease during greening (1, 4, 20). Cyt *f* synthesis, however, differed from the situation found in etioplast to chloroplast transformations; the synthesis of Cyt *f* during Fe resupply was rapid, so the Chl/Cyt *f* ratio declined (cf P_{700}). In the etioplast system, the Chl to Cyt *f* ratio increases hyperbolically during the greening process due to the fact that etioplasts contain a significant amount of Cyt *f* (5, 6, 13, 20).

The depression in Chl/P_{700} and Chl/Cyt *f* after 48 h of Fe resupply appeared to qualitatively correlate with the photosynthetic rate/Chl, *i.e.* photosynthesis/Chl attained the highest value at 48 h, the time when the Chl/P_{700} and Chl/Cyt *f* were lowest in experiment 1. This suggests that light-saturated photosynthesis depended on the concentration of reaction centers (P_{700}) and electron carriers (Cyt *f*) rather than on Chl content.

Chl *b* has been associated with the light-harvesting complex and with the formation of grana stacks (2, 7, 9, 11, 22, 30). Thus, the increase in Chl *b* which occurred after 48 h may be the point at which grana stacks begin to develop in Fe-resupplied plants. This is qualitatively supported by the electron microscopy of Platt-Aloia *et al.* (19), which showed that there was a lag phase of approximately 48 h in grana formation.

Several studies have indicated that chloroplasts with decreased grana have increased Q/P_{700} ratios. This has been shown for developing plants and mutants incapable of complete chloroplast development (16, 17). Similarly, during Fe resupply the Q/P_{700} ratio decreased from 2.55 after 24 h to 0.73 after 96 h, over which time granal development occurs (19).

The regreening of chlorotic leaves by resupplying Fe to Fe-deficient plants is usually attributed to the role of Fe in Chl synthesis (see (8)). When Fe was resupplied, the components of electron transport severely affected by Fe deficiency, *i.e.* Cyt *f* and P_{700}, were rapidly synthesized, followed later by increased synthesis of Q and the bulk Chl. These results indicate that Fe-mediated chloroplast development is regulated and that the role of Fe extends beyond that of Chl synthesis. Fe may affect (either directly or indirectly) the synthesis of some electron transport components, proteins (as suggested by Jacobson and Oertli [14]) and/or other membrane components such as lipids. Interestingly, Funkhouser and Price (10) found half as much chloroplast RNA and chloroplast ribosomes in Fe-deficient *Euglena* as in controls, while cytoplasmic RNA was unaffected.

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