Plant Physiol. (1985) 78, 296–299
0032-0889/85/78/0296/04/$01.00/0

Chlorophyll-Proteins and Electron Transport during Iron Nutrition-Mediated Chloroplast Development

Received for publication December 18, 1984

JOHN N. NISHIO,[1] JAVIER ABADÍA,[2] AND NORMAN TERRY*
Department of Plant and Soil Biology, University of California, Berkeley, California 94720

ABSTRACT

Chlorophyll-protein complexes and electron transport activities were measured during iron nutrition-mediated chloroplast development in sugar beet (*Beta vulgaris* L. cv F58-554H1). Results showed that the chlorophyll-protein complexes associated with the reaction centers of photosystem I (CP1) and photosystem II (CPa) and the electron transport activities of these two photosystems per leaf area increased rapidly during the first 24 to 48 hours of iron resupply to iron-deficient sugar beet plants. Bulk chlorophyll and the amounts of light-harvesting chlorophyll-proteins increased after a lag period of 24 hours. The changes in chlorophyll-proteins with time were apparently the cause of an initial increase, then decrease, in the chlorophyll *a/b* ratio during iron resupply. There was evidence that iron deficiency diminished photosystem I more than photosystem II. We propose that there are two distinct phases in iron nutrition-mediated chloroplast development: (a) the commencement of the synthesis of the lipid matrix of the thylakoid membrane, including a fully functioning electron transport (and photosynthetic) system, during the first 24 hours of iron resupply; and (b) after 24 to 48 hours, the formation of the bulk of the thylakoid proteins, including the light-harvesting chlorophyll-proteins with which the large increase in total chlorophyll is associated.

Earlier work showed that when Fe was resupplied to Fe-deficient sugar beet plants, the synthesis of thylakoid membrane lipids (galactolipids) was linear over a 96-h period (12). The PSI electron transport components, P_{700} and Cyt *f*, also increased linearly with time (13). However, total Chl and total thylakoid protein exhibited a lag of 24 h before maximum rates of synthesis were attained (12). Chl *a/b* ratios have been shown to increase over the first 24 h of resupply, then decrease (12, 13, 17).

These experimental observations suggest that during Fe nutrition-mediated chloroplast development, the membrane matrix is laid down first with reaction centers and electron carriers, and that after 24 h there is increased synthesis of the LHCPs[3] (majority of Chl) and other thylakoid proteins. Thus, during the first 24 h, the reaction center Chl-proteins (mainly Chl *a*-containing) would be accumulated more rapidly than the Chl *b* (and Chl *a*) containing LHCPs. Thereafter, the LHCPs would (according to this view) accumulate faster than the reaction centers, and the Chl *a/b* ratio would decrease to control levels.

The objective of the present paper was to test this hypothesis by determining the changes in electron transport activities and Chl-protein complexes following Fe resupply to Fe-deficient plants. Short, nondenaturing gel electrophoresis was utilized to specifically examine the reaction center containing Chl-proteins and LHCPs during greening. In addition, the rate of photosynthetic O_2 evolution was measured using leaf slices to determine the extent to which photosynthetic rate paralleled the changes in photosynthetic electron transport.

MATERIALS AND METHODS

Plant Culture. Sugar beets (*Beta vulgaris* L. cv F58-554H1) were cultured, made iron deficient, resupplied with iron, and harvested as described previously (12).

Thylakoid Extraction. For gel electrophoresis, thylakoids were extracted as previously described (12) and stored in 50 mM Tricine, pH 8.0 (1). For electron transport, thylakoids were extracted by the method of Ball *et al.* (4) except that chloroplasts were not osmotically shocked. To obtain thylakoids, chloroplasts in resuspension buffer were homogenized in an electrically powered Kontes Teflon homogenizer. The homogenized chloroplast suspension was centrifuged at 12,000*g* for 3 to 5 min. The homogenization and centrifugation were repeated and the pellet was brought up to desired volume in the 400 mM sorbitol buffer solution.

Electron Transport. Electron transport measurements were made with a Rank O_2 electrode using the procedure of Ball *et al.* (4). PSI activity was measured as O_2 consumption (ascorbate-DCIP → MV). PSII activity was measured as O_2 evolution (H_2O → pBQ) and PSI + PSII activity was measured as O_2 consumption (H_2O → MV).

Gel Electrophoresis. Slab gels were run according to Anderson *et al.* (2). Because of the higher protein to Chl ratio in chlorotic tissue (12), the following SDS:Chl ratios were utilized to obtain complete solubilization: 48 and 96 h, 7; 24 h, 12; and 0 h, 25. The electrophoretic pattern of thylakoids of Fe-deficient plants resupplied with Fe for 96 h is similar to that for thylakoids of Fe-sufficient plants (*i.e.* control plants) (12).

Leaf Slice Photosynthesis. Leaf slice photosynthesis was measured with a Rank O_2 electrode according to Papp *et al.* (15). Leaf slices (1 cm × 0.42 mm) were cut with a Spencer '820' microtome.

Chl. Chl was determined in 80% acetone according to Arnon (3).

RESULTS

Iron deficiency diminished the rate of PSI electron transport per leaf area to about 12% of the control; when Fe was resupplied to Fe-deficient plants, PSI activity returned to control levels after

[1] Present address: Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

[2] Recipient of a postdoctoral fellowship from the Spanish Superior Council of Scientific Research (C.S.I.C.). Permanent address: Estación Experimental de Aula Dei (C.S.I.C.), Apartado 202, Zaragoza, Spain.

[3] Abbreviations: LHCP, light-harvesting Chl-protein; CP; Chl-protein; DCIP, 2,6-dichlorophenolindophenol; MV, methyl viologen; pBQ, *p*-benzoquinone.

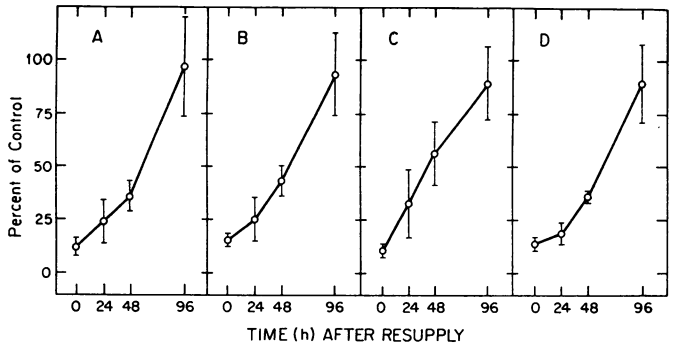

FIG. 1. Changes in electron transport activity and Chl based on leaf area (% of control) during Fe nutrition-mediated chloroplast development. A, PSI activity (ascorbate-DCIP → MV); B, PSII activity (H_2O → pBQ); C, whole chain electron transport activity (H_2O → MV); D, Chl content. Each point is the mean of at least three measurements (± SE) of pooled samples of five or six leaves per sample. Mean (± SE) control values (n = 12) were 46.8 ± 4.7 for PSI, 18.7 ± 2.6 for PSII, and 13.0 ± 1.5 for whole chain activity (mmol O_2 mol Chl^{-1} s^{-1}).

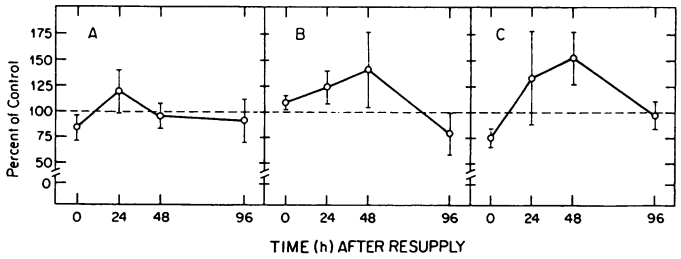

FIG. 2. Changes in electron transport on a per Chl basis during iron nutrition-mediated chloroplast development. A, PSI activity as a per cent of control; B, PSII activity as a per cent of control; C, whole chain activity as a per cent of control. Each point is the mean of at least three measurements (± SE) of samples composed of at least five leaves per sample. Control values are given in Figure 1.

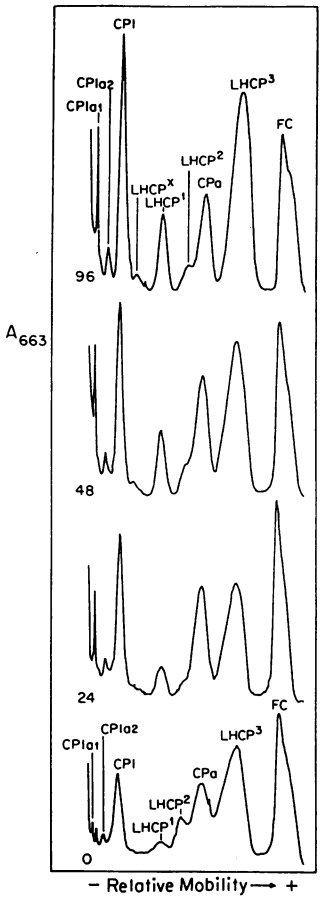

FIG. 3. Densitometer traces (A_{663}) (10) of green gels run according to Anderson *et al.* (2). Chlorotic tissue (0 h) is at bottom and 96 h after resupply of iron is at the top.

96 h (Fig. 1). PSII activity was affected similarly by Fe deficiency, decreasing to 15% of the control; it increased to about 95% after 96 h of regreening. The rate of whole chain electron transport per leaf area was reduced to a level comparable to that of PSI, about 11% of the control, and increased to 90% of the control after 96 h of Fe resupply. By comparison, Fe deficiency decreased the Chl content per leaf area to 14% of the control (Fig. 1D). As in earlier experiments (12, 13), Chl synthesis exhibited a distinct lag phase during the first 24 h of regreening, eventually reaching 90% of the control by the end of the experiment.

When electron transport activities were expressed on a Chl basis, PSI activity increased to 119% of the control during the first 24 h of Fe resupply, then decreased to slightly less than control levels by 96 h of regreening (Fig. 2A). PSII activity per Chl increased during the first 48 h, then decreased to about 80% of control levels (Fig. 2B). Rates of whole chain electron transport increased to 150% of the control after 48 h of regreening and returned to control levels by 96 h (Fig. 2C).

The pattern of separation of the Chl-proteins was similar to that obtained by Anderson *et al.* (2) and their nomenclature is used to describe the Chl-proteins. The PSI-associated Chl-proteins are represented by CP1, CP1a1, and CP1a2, and the PSII reaction center containing Chl-protein is represented by CPa. The light harvesting complexes were separated into four bands—$LHCP^1$, $LHCP^2$, $LHCP^3$, and $LHCP^x$.

The percentage of Chl associated with each Chl-protein was calculated from the area under the curves on gel scans similar to the one in Figure 3. The ΣCP1 Chl (CP1a1 + CP1a2 + CP1) was 14% of the total Chl in Fe-deficient plants. After 24 h of regreening, ΣCP1 Chl increased to 22% and remained at that level (Fig. 4A). The proportion of the total Chl associated with CPa, which was 14% in Fe-deficient plants, increased to 17% after 24 h, then decreased to 12% after 96 h of Fe resupply (Fig. 4B).

The proportion of the total Chl associated with LHCP (ΣLHCP) decreased from 41% in Fe-deficient plants to 33% during the first 24 h of resupply, then returned to 44% over the remainder of the 96 h regreening period (Fig. 4C). The ratio of (ΣCP1 Chl)/(CPa Chl) was less than 1 in chlorotic tissue and increased to more than 1.75 after 96 h of resupply (Fig. 4D). About 25% of the total Chl was free pigment in Fe-deficient plants, and 20% in resupplied plants. The large amount of free Chl was due to the longer gel runs required to separate the bands in Fe-deficient tissue (Fig. 3). The need for a longer run may have been due to the greater amount of protein loaded for chlorotic samples, as the Chl/protein ratio is elevated in iron-deficient plants (12).

The rate of photosynthetic O_2 evolution of leaf slices expressed on a per leaf area basis was 10% of the control rate in Fe-deficient plants (Fig. 5A). After 96 h of Fe resupply, it increased to 81% of control. When expressed per Chl, the rate increased to about 175% of control 48 h after Fe resupply, then decreased to control levels after 96 h (Fig. 5B).

DISCUSSION

Earlier work showed that one of the first events in the development of thylakoid membranes following Fe resupply to Fe-deficient sugar beets is the formation of membrane galactolipids;

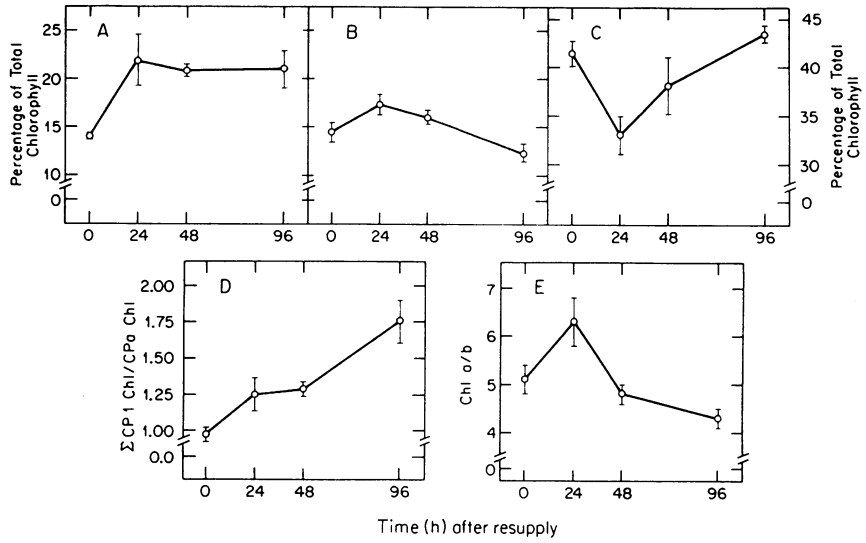

FIG. 4. Changes in the percentage of total Chl associated with various Chl-proteins after resupply of Fe to Fe-deficient plants. A, ΣCP1; B, CPa; C: ΣLHCP; D, ratio of (ΣCP1 Chl)/(CPa Chl); E, Chl *a*/Chl *b* ratio. Each point is the mean (± SD) of approximately five replicates from each of two pooled leaf samples (five or six leaves per sample).

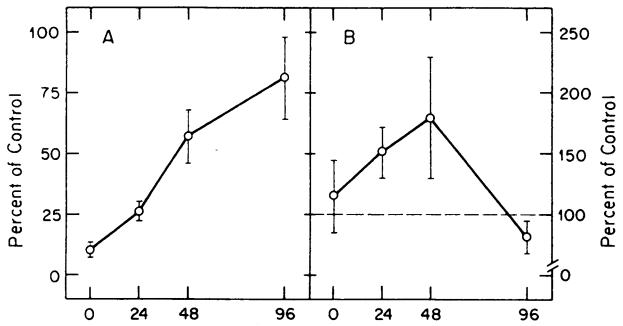

FIG. 5. Changes in leaf slice photosynthesis after resupply of Fe to Fe-deficient sugar beet plants. A, Leaf area basis; B, Chl basis. Each point is the mean of five or six measurements (± SE). Control values were 5.5 ± 0.6 mmol O_2 mol Chl^{-1} s^{-1}; 3.2 ± 0.3 μmol O_2 m^{-2} s^{-1}.

this is followed about 24 h later by the synthesis of the bulk of the thylakoid proteins (12). The results of the present work show that the rudiments of a functioning electron transport system were also laid down early in the development of these membranes. PSI, PSII, and whole chain electron transport activities developed during the first 24-h period of Fe resupply, as did the Chl-proteins associated with the reaction centers of PSI and PSII, CP1, and CPa, respectively.

The results of the Chl-protein analysis appear to explain the initial increase (over the first 24 h), then decrease, in the Chl *a*/*b* ratio which has been observed before (12, 13, 17) and in the present work (Fig. 4E). These changes apparently occur because the percentage of Chl associated with the Chl *a*-containing reaction center Chl-proteins, CP1 and CPa, increased during the first 24 h of resupply while the percentage associated with the LHCPs (which contains Chl *b* and *a*) decreased; hence, the increase in the Chl *a*/*b* ratio during the first 24 h of Fe resupply. Subsequently, there was a decrease in the Chl *a*/*b* ratio as the proportion of LHCP increased relative to the reaction center Chl proteins.

Iron deficiency appeared to diminish PSI constituents more than PSII. For example, the ratio of CP1/CPa was significantly decreased by Fe deficiency. In an earlier study P_{700}/Q ratios were found to be lower in Fe-deficient leaves suggesting that there were fewer PSI than PSII reaction centers (13). Research with cyanobacteria has also indicated that Fe deficiency causes a decrease in PSI components, *e.g.* P_{700} and membrane-bound Fe-S centers (8, 14, 16).

Not only was PSI decreased more by Fe deficiency than PSII, it also appeared to increase more rapidly than PSII during Fe resupply. This was indicated by the increase in the ratio of CP1/CPa from <1.0 to 1.75 after 96 h of regreening (Fig. 4D). The data suggest that the rate of PSI electron transport per Chl increased over the first 24 h, then remained near control levels, while PSII activity per Chl peaked later, at 48 h (Fig. 2). These data correspond to earlier data which show that during the early regreening period the ratios of P_{700}/Chl and Cyt *f*/Chl increased more rapidly than Q/Chl and that the Q/Chl ratio was highest after 24 h regreening and lowest in green tissue (13).

The early establishment of a functioning electron transport apparatus in Fe nutrition-mediated chloroplast development was accompanied by a rapid increase in the photosynthesis of leaf slices (Fig. 5). The rate of photosynthetic O_2 evolution per Chl paralleled the changes in the rates of electron transport per Chl which increased over the first 48 h, then decreased to control levels. Similar results were obtained with the rate of photosynthetic CO_2 fixation measured by gas exchange at light saturation and normal ambient CO_2 levels (13). These data highlight the importance of the electron transport system as a rate-limiting factor of photosynthesis (19, 20).

The absence of any significant effect of Fe deficiency on PSI and PSII electron transport activities and leaf slice photosynthesis (expressed per Chl) is in agreement with electron transport measurements in cyanobacteria (7). It is also consistent with other reports that Fe deficiency has no effect on rates of photosynthesis/Chl (5, 18, 20). However, there are studies showing that Fe deficiency results in increased Chl *a*/*b* ratios and in increased rates of photosynthesis/Chl (11). One explanation for the latter result is that Fe-deficient plants may in some instances undergo physiological or environmental changes resulting in some resupply of Fe so that the plants correspond to the early Fe resupply condition as typified in our work.

Research conducted using the Fe nutrition-mediated chloroplast development system suggests that the formation of thylakoid membranes during Fe resupply is a well-orchestrated process, and supports the stepwise theory of membrane development. There is clear evidence of at least two distinct phases of development. In the initial phase, a lipid matrix, which includes a rudimentary and fully functioning electron transport system, is synthesized during the first 24 h of regreening. In the second phase, after about 24 to 48 h of resupply, the bulk Chl (LHCPs) and the majority of thylakoid proteins accumulate.

The appearance and disappearance of specific thylakoid proteins and Chl-proteins during Fe resupply indicates that Fe nutrition-mediated chloroplast development is a well-regulated

process (13). In Fe-deficient plants, chloroplast rRNA is decreased to a greater extent than cytoplasmic rRNA (6, 9). On resupply of Fe, chloroplast rRNA synthesis exhibits a lag of 12 h (9). This observation is in accordance with the 24 h lag in total thylakoid protein synthesis which occurred in our studies. In conclusion, we believe that Fe nutrition-mediated chloroplast development provides a useful and practical method for studying *de novo* synthesis of photosynthetic membranes.

Acknowledgments—We thank Dr. Lowell Lewis for use of equipment, and Mr. Clifford Carlson for culturing the plants.

LITERATURE CITED

1. ABADÍA J, JN NISHIO, N TERRY 1985 Chl-protein and polypeptide composition of Mn-deficient sugar beet thylakoids. Photosyn Res. In press
2. ANDERSON JM, JC WALDRON, SW THORNE 1978 Chlorophyll-protein complexes of spinach and barley thylakoids. FEBS Letters 92: 227–233
3. ARNON DI 1979 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
4. BALL MC, SE TAYLOR, N TERRY 1984 A comparative study of photosynthetic electron transport in the mangroves, *Avicennia germinans* and *Avicennia marina*, and the sugar beet, *Beta vulgaris*, in response to salinity. Plant Physiol 76: 531–535
5. BOTTRILL DE, JV POSSINGHAM, PE KRIEDEMANN 1970 The effect of nutrient deficiencies on photosynthesis and respiration in spinach. Plant Soil 32: 424–438
6. FUNKHOUSER EA, CA PRICE 1974 Chloroplast RNA: possible site of an early lesion in iron deficiency. Plant Cell Physiol 15: 883–889
7. GUIKEMA JA, LA SHERMAN 1983 Organization and function of chlorophyll in membranes of cyanobacteria during iron starvation. Plant Physiol 73: 250–256
8. HARDIE LP, DL BALKWILL, SE STEVENS JR 1983 Effects of iron starvation on the physiology of the cyanobacterium *Agmenellum quadruplicatum*. Appl Environ Microbiol 45: 999–1006
9. LAULHÈRE J-P, R MACHE 1978 Induction des synthèses de mRNA et rRNA chloroplastique par le fer lors du verdissement de feuilles chlorosées. Physiol Veg 16: 643–656
10. LICHTENTHALER HK, G BURKHARD, G KUHN, U PRENZEL 1981 Light-induced accumulation and stability of chlorophylls and chlorophyll-protein complexes during chloroplast development in radish seedlings. Z Natursforch 36C: 421–430
11. NAIK GR, GV JOSHI 1979 Photosynthetic carbon fixation in iron-chlorotic and recovered green sugarcane leaves. Plant Soil 53: 505–511
12. NISHIO JN, SE TAYLOR, N TERRY 1985 Changes in thylakoid galactolipids and protein during iron nutrition-mediated chloroplast development. Plant Physiol 77: 705–711
13. NISHIO JN, N TERRY 1983 Iron nutrition-mediated chloroplast development. Plant Physiol 71:688–691
14. ÖQUIST G 1974 Iron deficiency in the blue-green alga *Anacystis nidulans*: Changes in pigmentation and photosynthesis. Physiol Plant 30: 30–37
15. PAPP JC, MC BALL, N TERRY 1983 A comparative study of the effects of NaCl salinity on respiration, photosynthesis, and leaf extension growth in *Beta vulgaris* L (sugar beet). Plant Cell Environ 6: 675–677
16. SANDMANN G, R MALKIN 1983 Iron-sulfur centers and activities of the photosynthetic electron transport chain in iron-deficient cultures of the blue-green alga *Aphanocapsa*. Plant Physiol 73: 724–728
17. SHETTY AS, GW MILLER 1966 Influence of iron chlorosis on pigment and protein metabolism in leaves of *Nicotiana tabacum* L. Plant Physiol 41: 415–421
18. SPENCER D, JV POSSINGHAM 1960 The effect of nutrient deficiencies on the Hill reaction of isolated chloroplasts from tomato. Aust J Biol Sci 13: 441–455
19. TAYLOR SE, N TERRY 1984 Limiting factors in photosynthesis. V. Photochemical energy supply colimits photosynthesis at low values of intercellular CO_2 concentration. Plant Physiol 75: 82–86
20. TERRY N 1983 Limiting factors in photosynthesis. IV. Iron stress-mediated changes in light harvesting and electron transport capacity and its effect on photosynthesis *in vivo*. Plant Physiol 71: 855–860