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Short Communication

Differential Changes in the Amount of Protein Complexes in the Chloroplast Membrane during Senescence of Oat and Bean Leaves

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ABSTRACT

Antibodies against the individual subunits of protein complexes in the chloroplast membranes were used to follow the amounts of these polypeptides during foliar senescence. No change was found in the amount of polypeptides of photosystem I reaction center and the chloroplast coupling factor during senescence of oat (*Avena sativa* L.) and bean (*Phaseolus vulgaris* L.) leaves. A significant decrease in the amount of the different components of the cytochrome b_6-f complex was detected. This change may account for the decrease in the rate of electron transport, which might be the rate limiting step of photosynthesis in senescing leaves.

Foliar senescence is characterized by a major change in the chloroplast structure and function (2, 5). Most studies on senescing chloroplasts were based upon *in vitro* measurements of partial photosynthetic reactions. Examples are CO_2 fixation (7, 11), photophosphorylation (11, 13), Hill reaction (10), and electron transport through PSI and PSII (4, 7, 13, 14, 19). Although a decrease in the rate of some of these reactions was demonstrated, there was no definite identification of a change in a specific site(s) that might limit the rate of photosynthesis during senescence.

Electron microscopy revealed a decrease in plastid volume (2) and a disruption of the internal membrane system in chloroplasts from senescing leaves (5). Biochemical studies showed that along with these morphological changes, there is a change in the lipid composition of the membranes during senescence (2). These findings may imply that the changes in structure and physical properties of the thylakoid membranes coincide with the decrease in the rate of photosynthetic activity. In the present study, an immunological approach was applied in order to follow the changes in the relative amounts of some of the chloroplast membrane protein complexes during senescence.

MATERIALS AND METHODS

Leaves. *Phaseolus vulgaris* L. cv Brittlewax (bean) and *Avena sativa* L. cv Victory (oat), representing two different plant categories, were grown in vermiculite under controlled growth room conditions. Temperature was maintained at 24°C and the intensity of illumination employed for 12 h/d was about 1.5×10^3 erg/cm^2·s. Primary leaves were harvested after full expansion was achieved (15 and 8 d after sowing bean and oat seeds, respectively).

Controlled Facilitated Senescence. Three-cm-long segments were cut off the tips of 8-d-old oat leaves, and then incubated on wet gauze for 4 d at 24°C in the dark.

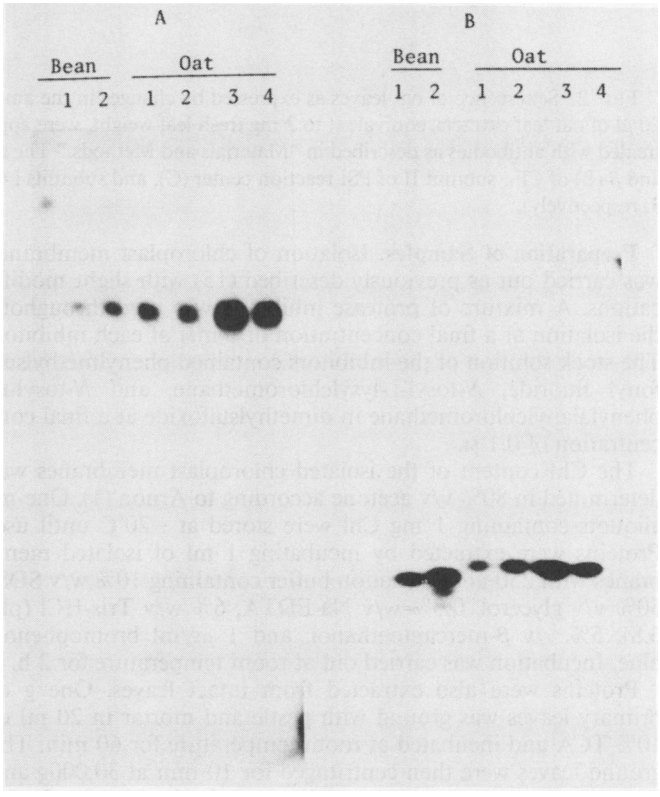

FIG. 1. Changes in the amount of PSI reaction center during senescence in bean and oat leaves. Samples of bean and oat leaf extracts were applied to SDS-polyacrylamide gels, transferred to nitrocellulose paper, and treated with antibodies as described in "Materials and Methods." Antibodies used were raised against subunits I (A) and II (B) of PSI reaction center. (1), Samples of 15-d-old bean leaf or 8-d-old oat leaf extracts containing 5 μg Chl. (2), Samples of 30-d-old bean leaves or 15-d-old oat leaf extracts containing 5 μg Chl. (3 and 4), Samples of oat leaf segments subjected to controlled facilitated senescence, containing 12 and 6 μg Chl, respectively.

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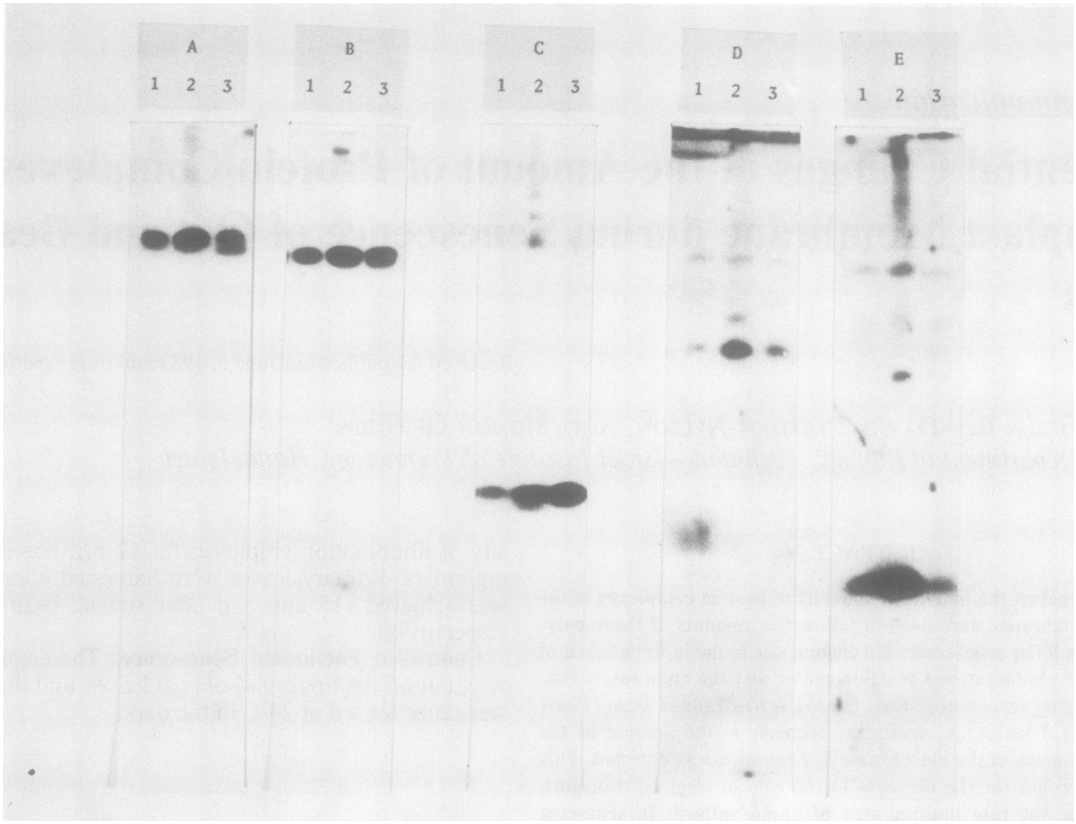

FIG. 2. Senescence of oat leaves as expressed by changes in the amount of protein complexes in the chloroplast membrane. Samples containing 20 μl of oat leaf extracts, equivalent to 2 mg fresh leaf weight, were applied to SDS-polyacrylamide slab gels, transferred to nitrocellulose paper, and treated with antibodies as described in "Materials and Methods." The antibodies used were raised against the following polypeptides: Subunits α (A) and β (B) of CF_1, subunit II of PSI reaction center (C), and subunits I (D) and IV (E) of the b_6-f complex. Leaves were 8, 15, and 30 d old (1, 2, and 3, respectively).

Preparation of Samples. Isolation of chloroplast membranes was carried out as previously described (15) with slight modifications. A mixture of protease inhibitors was used throughout the isolation at a final concentration of 1 mM of each inhibitor. The stock solution of the inhibitors contained phenylmethylsulfonyl fluoride, *N*-tosyl-L-lysylchloromethane, and *N*-tosyl-L-phenylalanylchloromethane in dimethylsulfoxide at a final concentration of 0.1 M.

The Chl content of the isolated chloroplast membranes was determined in 80% v/v acetone according to Arnon (1). One-ml aliquots containing 1 mg Chl were stored at −20°C until use. Proteins were extracted by incubating 1 ml of isolated membranes with 250 μl dissociation buffer containing 10% w/v SDS, 50% v/v glycerol, 0.3% w/v Na-EDTA, 6% w/v Tris-HCl (pH 6.8), 5% v/v β-mercaptoethanol, and 1 μg/ml bromophenol-blue. Incubation was carried out at room temperature for 2 h.

Proteins were also extracted from intact leaves. One g of primary leaves was ground with pestle and mortar in 20 ml of 10% TCA and incubated at room temperature for 60 min. The ground leaves were then centrifuged for 10 min at 30,000*g* and the pellet was resuspended and homogenized with 10 ml of a solution containing 100 mM Tris-HCl (pH 8.0) and 100 μl of the protease inhibitor mixture. Two g glass beads (1 mm in diameter) were added and the homogenate was vortexed vigorously for 30 s. Acetone was added to give a final concentration of 80% v/v and, after 5 min incubation at room temperature, the extract was centrifuged for 10 min at 30,000*g*. The pellet was washed twice with 10 ml of 80% v/v acetone as described above. The final pellet was dried by a stream of N_2 gas and resuspended in 8 ml of a solution containing 2% w/v SDS and 80 μl of the protease inhibitor mixture. After 20 min incubation at room temperature, the suspension was centrifuged for 10 min in an Eppendorf microfuge and the supernatant was stored at −20°C until use. One-ml samples for electrophoresis were incubated for 2 h at room temperature with 250 μl of the same dissociation buffer used for the isolated chloroplast membranes.

Published procedures were used for SDS-polyacrylamide gel electrophoresis (8), electrotransfer to nitrocellulose papers (20), and immunodecoration with antibodies and ^{125}I-protein A (17). The immunodecorated nitrocellulose papers were exposed to x-ray films.

Antibodies. Antibodies against the individual subunits of protein complexes from spinach chloroplast membranes were raised in rabbits as previously described (17). A cross-reactivity between antibodies raised against antigens isolated from spinach and the same polypeptides from oat and bean leaves was found and is demonstrated in the text (Figs. 1–3).

RESULTS AND DISCUSSION

Figure 1 shows a small increase in the amounts of subunits I and II of PSI reaction center (3). However, the basis for comparison was the amount of Chl in the samples. Taking into consideration the decrease in the Chl content of the leaf during senescence (9, 13), this phenomenon reflects a small, if any, change in the amount of PSI reaction center during senescence. When the same comparison was made on the basis of fresh leaf weight, no change in the amount of PSI reaction center could be detected (Fig. 2C).

As shown in Figure 2, A and B, there is no detectable decrease

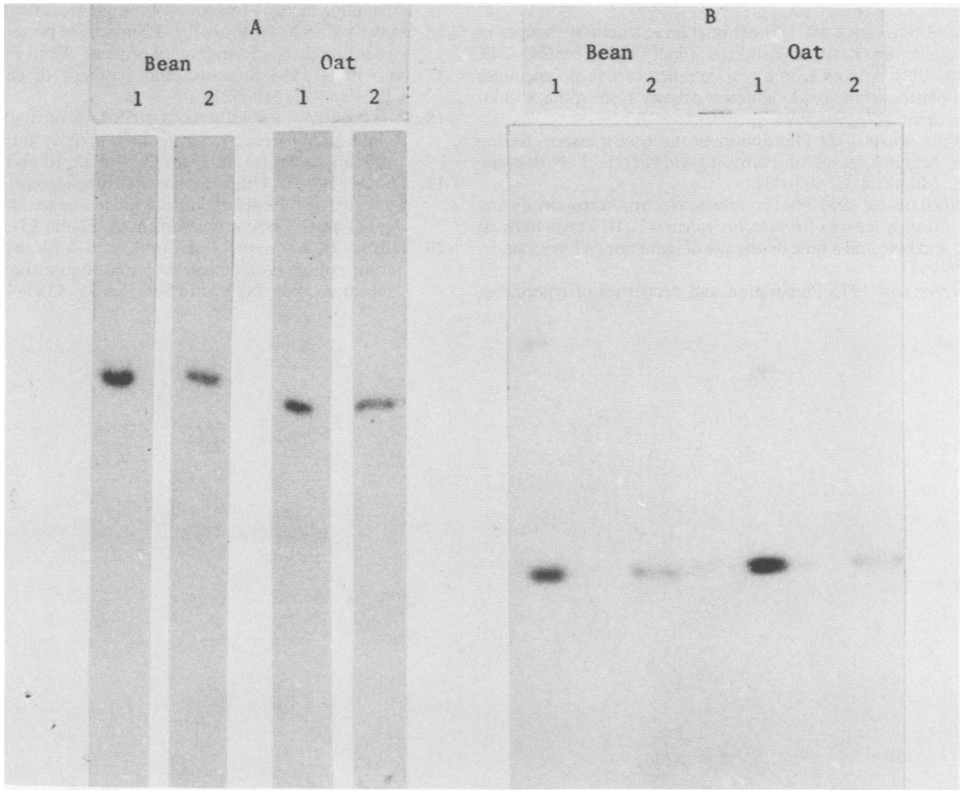

FIG. 3. Changes in the amount of b_6-f complex during senescence in bean and oat leaves. Samples from bean and oat leaves extracts containing 10 μg Chl were applied to SDS-polyacrylamide gels, transferred to nitrocellulose paper, and treated with antibodies as described in "Materials and Methods." Antibodies were raised against subunits I (A) and IV (B) of the b_6-f complex. Bean leaves were 15 (1) and 30 (2) d old and oat leaves were 15 (1) and 30 (2) d old.

in the amount of subunits α and β of CF_1[2] during foliar senescence. These findings are in line with the reports of Jenkins and Woolhouse (13) on a constant P/2e ratio obtained in chloroplasts isolated from senescing bean leaves. Our results are in contrast to observations reported by Camp *et al.* (6), demonstrating a loss of CF_1 during senescence. This contradiction can be explained by the effect of endogenous proteases released during the isolation of membranes, and by a lack of specificity of the procedures employed on those studies. In the present study, the endogenous proteases, known to be synthesized and activated during senescence (16, 18), were inhibited soon after leaves were harvested, by incubation with TCA and by the use of protease inhibitors.

In contrast to the above-mentioned findings, a pronounced decrease in the amount of Cyt b_6-f complex was detected during foliar senescence. The dramatic decrease in the amounts of subunits I and IV of Cyt b_6-f complex was noticeable during the period between 15 and 30 d after sowing (Fig. 3) on the basis of Chl content, and Figure 2, D and E, shows the same on the basis of fresh leaf weight. The decrease in the amount of the Cyt b_6-f complex is detected before the decay in the Chl content of the leaf starts (9, 13).

As evident from the observations, the ratio between the amounts of all subunits of a single protein complex remains fairly constant during senescence. Accordingly, it is possible to learn about the changes a certain protein complex undergoes during senescence by following a representative subunit.

The detected decrease in the amount of the b_6-f complex subunits could not be explained as previously suggested (13) by their release from the chloroplast membranes, since their amount during senescence decreases not only in isolated membranes but also in the crude leaf extract. A possible explanation is a change in the rate of synthesis and degradation of these polypeptides taking place during senescence.

Jenkins and Woolhouse (14) found a small decrease in the activity of PSI and PSII that could not account for the large decrease in the rate of electron transport during foliar senescence. They suggested that the rate limiting step lies somewhere in between the two photosystems. According to our results, a possible candidate for this limitation is the Cyt b_6-f complex. The decrease in its relative amount serves as a cause for the decrease in the rate of electron transport that might, in turn, limit the rate of photosynthesis in the senescing leaf.

[2] Abbreviation: CF_1, chloroplast coupling factor.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in chloroplasts. Polyphenyloxidases in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BARTON R 1966 Fine structure of mesophyll cells in senescing leaves of *Phaseolus*. Planta 71: 314–325
3. BENGIS, C, N NELSON 1975 Purification and properties of photosystem I reaction center from chloroplasts. J Biol Chem 250: 2783–2788
4. BISWAL UC, P MOHANTY 1976 Aging induced changes in photosynthetic electron transport of detached barley leaves. Plant Cell Physiol 17: 323–331
5. BUTLER RD, EW SIMON 1970 Ultrastructural aspects of senescence in plants. Adv Gerontol Res 3: 73–129
6. CAMP PJ, SC HUBER, JJ BURK, DE MORELAND 1982 Biochemical changes that occur during senescence of wheat leaves. I. Basis for the reduction of photosynthesis. Plant Physiol 70: 1641–1646
7. CHOE HT, KV THIMANN 1977 The retention of photosynthetic activity by senescing chloroplasts of oat leaves. Planta 135: 101–107
8. DOUGLASS MG, RA BUTOW 1976 Variant forms of mitochondrial translation products in yeast evidences for location on mitochondrial DNA. Proc Natl Acad Sci USA 73: 1083–1086
9. GEPSTEIN S, KV THIMANN 1980 Changes in the abscisic acid content of oat leaves during senescence. Proc Natl Acad Sci USA 77: 2050–2053
10. HARNISCHFEGER G 1974 Studies on chloroplast degradation in vivo. II. Effect of aging on Hill activity of plastids from Cucurbita cotyledons. Z Pflanzen-

physiol 71: 301–302
11. HERNANDEZ-GIL R, M SCHAEDLE 1973 Functional and structural changes in senescing *Populus deltoides* (Bartr.) chloroplasts. Plant Physiol 51: 245–249
12. HURT E, G HAUSKA 1981 A cytochrome f/b_6 complex of five polypeptides with plastoquinol-plastocyanine-oxidoreductase activity from spinach chloroplasts. Eur J Biochem 117: 591–599
13. JENKINS GI, HW WOOLHOUSE 1981 Photosynthetic electron transport during senescence of the primary leaves of *Phaseolus vulgaris* L. I. Non-cyclic electron transport. J Exp Bot 32: 467–478
14. JENKINS GI, HW WOOLHOUSE 1981 Photosynthetic electron transport during senescence of the primary leaves of *Phaseolus vulgaris* L. II. The activity of photosystems one and two, and a note on the site of reduction of ferricyanide. J Exp Bot 32: 989–997
15. KAMIENIEZKY A, N NELSON 1975 Preparation and properties of chloroplast coupling factor 1 by sodium bromide treatment. Plant Physiol 55: 282–287
16. MARTIN C, KV THIMANN 1972 The role of protein synthesis in the senescence of leaves. I. The formation of protease. Plant Physiol 49: 64–71
17. NELSON N 1983 Structure and synthesis of chloroplast ATPase. Methods Enzymol 97: 510–523
18. PETERSON LW, RC HUFFAKER 1975 Loss of ribulose-1,5-diphosphate carboxylase and increase in proteolytic activity during senescence of detached primary barley leaves. Plant Physiol 55: 1009–1015
19. THOMAS H 1977 Ultrastructure, polypeptide composition and photochemical activity in chloroplasts during foliar senescence of a non yellowing mutant genotype of *Festuca pratensis* huds. Planta 137: 53–60
20. TOWBIN H, T STAEHELIN, J GORDON 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350–4354