

Development of the Primary Photochemical Apparatus of Photosynthesis during Greening of Etiolated Bean Leaves¹

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ABSTRACT

Seven-day-old dark-grown bean leaves were greened under continuous light. The amount of chlorophyll, the ratio of chlorophyll *a* to chlorophyll *b*, the O₂ evolving capacity and the primary photochemical activities of Photosystem I and Photosystem II were measured on the leaves after various times of greening. The primary photochemical activities were measured as the photo-oxidation of P₇₀₀, the photoreduction of C-550, and the photo-oxidation of cytochrome *b*₅₅₉ in intact leaves frozen to -196 C. The results indicate that the reaction centers of Photosystem I and Photosystem II begin to appear within the first few minutes and that Photosystem II reaction centers accumulate more rapidly than Photosystem I reaction centers during the first few hours of greening. The very early appearances of the primary photochemical activity of Photosystem II was also confirmed by light-induced fluorescence yield measurements at -196 C.

It was apparent from the early studies of Smith (16) on the development of photosynthesis in etiolated leaves that the photosynthetic apparatus had to be assembled before the capacity for O₂ evolution could be realized. These early studies led to a number of subsequent investigations to correlate the appearance of photosynthetic capacities with the accumulation of Chl pigments and the development of specific structures associated with the photosynthetic apparatus. After it became apparent that the photosynthetic apparatus contained two spectrally distinguishable photochemical pigment systems the time course of the appearance of each of the two pigment systems, PSI and PSII, began to be investigated. The first of these investigations (6) using light-induced fluorescence yield changes to monitor PSI and PSII activity in intact etiolated bean leaves indicated that the two photosystems became functional in the first 2 to 3 hr of greening and that PSI was active before PSII. The same relative order of appearance of the two photosystems was reported by Gyldenholm and Whatley (9) with plastid preparations from the greening leaves using cyclic and noncyclic photophosphorylation to indicate PSI and PSII activity but they reported that these activities appeared only after 10 to 15 hr of greening. Similar requirements for rather long induction times (unreasonably long because it was known from the very early measurements of Smith that O₂ evolution began in earnest in intact leaves after about 2 hr of greening) were also reported by others who prepared plastids from the greening leaves for their assay systems (1, 13, 20). The question of the time of appearance of the

two photosystems was re-examined in intact etiolated leaves by Oelze-Karow and Butler (12) using assay procedures which measured photophosphorylation *in vivo*; PSI being measured as a DCMU-insensitive cyclic photophosphorylation and PSII as a DCMU-sensitive noncyclic photophosphorylation or as O₂ evolution. These studies on 8-day-old dark-grown bean leaves showed that noncyclic photophosphorylation and O₂ evolution both appeared at the same time after 1 hr of greening while cyclic photophosphorylation was present at 15 min which was the first assay time after the onset of greening. It was suggested (12) that in the previous studies with plastid preparations that the chloroplasts at early stages of development were inactivated by the isolation procedures. Later studies (10, 15) with improved plastid isolation techniques confirmed the early times of appearance of PSI and PSII activities reported by Oelze-Karow and Butler.

It is generally agreed that the electron transport activities associated with PSI appear in a greening leaf well before the PSII-mediated electron transfer reactions which lead to O₂ evolution. The time course of appearance of the primary photochemical apparatus of PSI and PSII, as noted by reaction center components, is less well established. Boardman *et al.* (3) reported that P₇₀₀ was not present in plastid preparations from etiolated pea seedlings during the first 3 hr of greening even though the PSI-mediated photo-oxidation of Cyt *f* was detected after 20 min. Plesnicar and Bendall (15) using plastid preparations from dark-grown barley leaves also reported that P₇₀₀ was absent during the 1st hr of greening while PSI activity was present and that C-550 appeared after about 2 hr of greening when O₂ evolution was first detected.

The purpose of the present work was to follow the development of the primary photochemical apparatus of PSI and PSII in intact dark-grown bean leaves during greening. The primary photochemical reactions of PSI and PSII were isolated from the secondary electron transport reactions by the simple procedure of freezing the leaves, at various stages of development, to liquid N₂ temperature. The primary photochemistry of PSI was measured as the photo-oxidation of P₇₀₀ at -196 C and the primary photochemistry of PSII as the photoreduction of C-550 with the accompanying oxidation of Cyt *b*₅₅₉. Light-induced fluorescence yield changes at -196 C were also used to monitor the primary photochemical activity of PSII.

MATERIALS AND METHODS

Bean seeds (*Phaseolus vulgaris* var. red kidney) were soaked in water for 18 hr and planted in coarse Vermiculite and the plants were grown in the dark at 25 C for 7 days. The plants were then allowed to green under a bank of eight 40 w Luxor Vita-lite tubes supplemented by eight 100 w tungsten lamps giving 8 mw/cm² of white light. After specific periods of greening the primary leaves were harvested from the plants for measurements.

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Total Chl of the leaf samples was determined by the method of Arnon (2); Chl *a*/Chl *b* ratios were determined in ethanolic extracts by the sensitive fluorometric method of Boardman and Thorne (4). Relative rates of O₂ evolution were measured with a 1-cm disc of the leaf placed directly on a Clark-type O₂ electrode (17). After a steady rate of O₂ uptake was established in darkness the sample was irradiated with broad band red light (50 mw/cm²) from a focused tungsten lamp:

Light-induced absorbance changes of P₇₀₀, C-550, and Cyt *b*₅₅₉ were measured on a double thickness of leaf (two 1-cm discs) frozen to -196 C. Absorption spectra were measured before and after a saturating irradiation with red light with our computerized single-beam spectrophotometer (7). The amount of P₇₀₀ was calculated as the absorbance difference between 702 and 690 nm, C-550 as the difference between 546 and 543 nm, and Cyt *b*₅₅₉ as the difference at 556 nm from an estimated base line on the light minus dark difference spectra.

Fluorescence yield changes were measured in leaf discs frozen to -196 C. Dual-arm fiber-optic light pipes were used to irradiate the top surface of the leaf and to transmit the fluorescence from the same surface to the phototube. Fluorescence was excited with broad band blue light (50 μw/cm²) and was measured with an EMI 9558 C phototube through a 695 nm interference filter (7 nm band pass) and a Corning 9830 filter. Care was taken to freeze the leaves in a dark-adapted state and to follow the transition from the minimal, *F*₀, level to the maximal, *F*_{*M*}, level. The fluorescence of variable yield was taken as the difference between *F*_{*M*} and *F*₀.

RESULTS

Time course curves for the accumulation of Chl, for the Chl *a*/*b* ratio, and for rates of O₂ evolution on a leaf area basis and on a Chl basis are shown in Figure 1 for 7-day-old dark-grown bean leaves. These results are in agreement with previous reports on the appearance of photosynthetic activity and Chl formation in etiolated leaves during greening.

Evidence for the appearance of the primary photochemical activity of PSI and PSII was sought by assaying for light-induced absorbance changes of reaction center components at -196 C. The extent of the light-induced absorbance changes of the P₇₀₀ of PSI and of the C-550 and Cyt *b*₅₅₉ of PSII are shown in Figures 2 and 3, respectively, at hourly intervals during the first 4 hr of greening. These types of data were used to plot time course curves for the accumulation of these components over the entire greening period (Fig. 4). It is apparent from extrapolations of the time course curves back toward zero time that active reaction centers of PSI and PSII begin to appear very soon (possibly a matter of minutes) after the onset of illumination. Figure 4 indicates that PSII reaction centers accumulate more rapidly

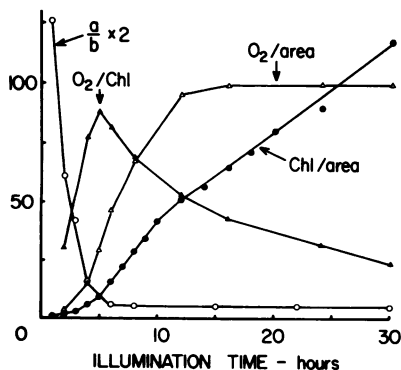


FIG. 1. Changes in the Chl content/unit leaf area ($\mu\text{g cm}^{-2}$), Chl *ab* ratio, photosynthetic O₂ evolution/unit leaf area and /unit Chl for bean leaves after various times of greening.

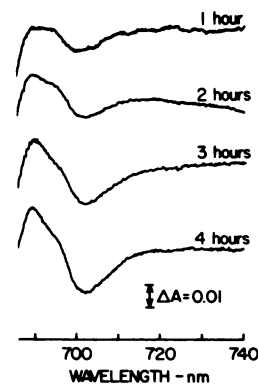


FIG. 2. Light minus dark difference spectra, measured at -196 C, showing the light-induced absorbance changes of P₇₀₀ occurring in bean leaves during the first 4 hr of greening.

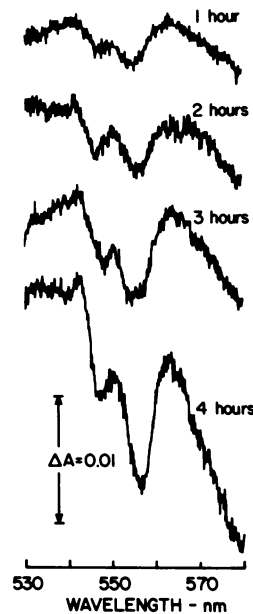


FIG. 3. Light minus dark difference spectra, measured at -196 C, showing the light-induced absorbance changes of C-550 and Cyt *b*₅₅₉ occurring in bean leaves during the first 4 hr of greening.

than PSI reaction centers (plotted as a percentage of the final value) during the first 4 hr of greening. The rate of P₇₀₀ accumulation may be limited during this period by the rate of Chl accumulation (Fig. 1) since both show a lag phase in the first 3 to 4 hr.

The early appearance of the primary activity of PSII was confirmed in measurements of the fluorescence of variable yield, *F*_{*V*}, at -196 C. At early stages of greening there is a large nonvariable component of fluorescence, *F*₀ at 695 nm due largely to the newly formed Chl that is not yet coupled to active PSII reaction centers. Any *F*_{*V*} at 695 nm, however, is due to Chl associated with PSII reaction centers. Figure 5 shows the ratio of *F*_{*V*}/*F*_{*M*} (where *F*_{*M*} = *F*₀ + *F*_{*V*}) measured at 695 nm from leaves frozen to -196 C after various times of greening. It is apparent that *F*_{*V*} appears very early after the onset of illumination and increases rapidly during the first 10 hr of greening.

DISCUSSION

Our results indicate that active PSI and PSII reaction centers both start to accumulate in etiolated leaves very soon after the onset of illumination. The very early appearance of the primary photochemical activity of PSII was not expected since measurements of photosynthetic electron transport activities at room

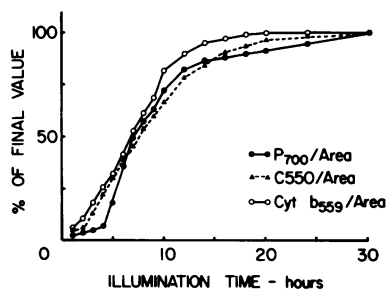


Fig. 4. Changes in the P_{700} , C-550, and Cyt b_{559} content/unit area of bean leaf after various times of greening.

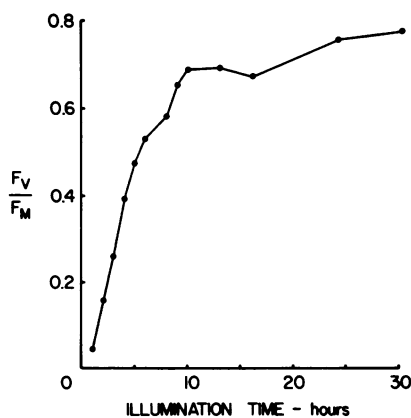


Fig. 5. Changes in the ratio of the fluorescence of variable yield to the maximal level of fluorescence (F_v/F_m), measured at 695 nm at 196 C, for bean leaves after various times of greening.

temperature had shown that PSI activity was present after 15 min of illumination while PSII activity was not present before 1 hr (12). We take our present results to indicate that the primary photochemical apparatus of both PSI and PSII begins to form within the first few min of illumination but that the electron transport chain needed for O_2 evolution is blocked at a secondary electron transfer reaction during the early times. Etiolated leaves which have been partially greened by a repetitive series of brief flashes show normal primary photochemical transformations of P_{700} and C-550 but are incapable of O_2 evolution due to a block of electron transport between water and PSII, probably at the site of Mn function (11, 14, 18, 19). This same block may prevent O_2 evolution during the early stages of greening of the dark-grown bean leaves as well.

We present our raw data (direct recordings of light minus dark difference spectra from the computer which takes the data) in Figures 2 and 3 to indicate the precision of our measurements of the light-induced absorbance changes of P_{700} , C-550, and Cyt b_{559} at -196 C because of the discrepancies with previous reports that these components were not present in plastid preparations at early stages of greening (4, 15). We found that measurements of light-induced absorbance changes at -196 C at early stages of greening were much more sensitive with intact leaf discs than with plastid preparations made from the leaves. Either the reaction center components tend to be inactivated by the plastid isolation procedures or the absorbance measurements are enhanced by greater light scatter in the intact leaves. We tend to favor the latter explanation and to expect that the intensification by light scatter should be greater at early stages of greening. The intensification of absorbance by light scatter, which is due to the greater optical path length, would be expected to decrease as the absorbance due to Chl increased (5). If we were to plot our data on the P_{700} and C-550 absorbance measurements (made on intact leaves) on a total Chl basis (determined from acetone extract of the leaves) we would find maximum values of P_{700}/Chl

and C-550/Chl ratios early in the greening regime which decrease markedly as the bulk of the Chl accumulates. However, we suspect that these early maximum values are due, at least in part, to the greater intensification of the absorbance measurements by light scatter in the intact leaves at the early stages of greening.

We can make some reasonable conjectures as to causative structure-function relationships during the early stages of the formation of the photosynthetic apparatus. At the onset of illumination Pchl is transformed to Chl and the crystalline array of tubules which makes up the prolamellar body breaks up into vesicles. PSI and PSII reaction centers start to form in these small Chl containing vesicles and the PSI-mediated cyclic photophosphorylation appears within the first few min (12). We infer from the photophosphorylation reaction that the vesicles contain an active proton pump. O_2 evolution, however, does not appear until after an hr of greening in normal, continuous visible light, during which time the vesicles coalesce into well formed primary thylakoids. It was noted previously (8) in greening experiments in far red light, where the developmental processes remain sequential and highly synchronized but much prolonged, that O_2 evolution does not appear until the primary thylakoids start to show well formed uniform membranes. At earlier stages the thylakoids are present but their membranes are uneven and possibly discontinuous. It is reasonable to suppose that sustained O_2 evolution requires effective ion pumps and the establishment of ion gradients so that the requirement for continuous well formed thylakoid membranes may be the necessity to isolate the intrathylakoid space from the stroma. However, experiments with leaves partially greened in a series of brief flashes have shown that well formed primary thylakoids are not a sufficient condition for O_2 evolution and that these thylakoids can be formed without functionality at the Mn site in the electron transport system between water and PSII. The terminal step in the formation of the capacity for O_2 evolution may be the photoactivation of Mn.

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