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Biosynthesis of the Photosystem I Chlorophyll–Protein Complex in Greening Leaves of Higher Plants

(photosystem I/chloroplast development/lamellar proteins/P700)

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ABSTRACT **Biosynthesis of photosystem I chlorophyll-protein complex, derived from chloroplast lamellae solubilized with Triton X-100, was examined in rapidly greening primary leaves of the jack bean. It was found that the chlorophyll/P700 ratio of the complex was low (35/1) in the early stages of greening, and increased steadily to a stable level (75/1) during continued chloroplast development. It is suggested from these results that in the initial stages of greening, and probably throughout synthesis, the P700–chlorophyll-protein molecules are first inserted into the developing lamellae, and those photosystem I chlorophyll-protein molecules functioning solely in a light-harvesting capacity are added subsequently.**

Information about the time course of biosynthesis of the photochemical apparatus of plants has been derived from examination of the activities of photosystems I and II during greening of dark-grown tissues (see refs. 1–3). Several investigators (4–11) have reported that photosystem I activity appears before that of photosystem II, while we (12) have observed the reverse order of biosynthesis in leaves greened under high relative humidity conditions and showing no lag in chlorophyll accumulation. The differences in results are most probably a reflection of environmental conditions during greening, rather than of species differences. It has been shown (13, 14) that low relative humidity conditions result in sufficient water stress to greening leaves to cause a lag in chlorophyll accumulation during the early stages of chloroplast development (see ref. 12). Under greening conditions with high relative humidity, however, the lag in chlorophyll accumulation is eliminated and there is rapid and uniform chloroplast development (12, 14, 15). In etiolated tissues greening under high relative humidity conditions, photosystem II becomes functional before photosystem I. Thus, it appears that assembly processes for photosystem II are more responsive to water stress than are those for photosystem I (16).

Further, our work (12) supports the generally accepted view (13, 17–20) that a step-wise, rather than a simultaneous, synthesis of the components of the thylakoid membranes occurs. We (12) detected the first signs of photosystem II activity after 2 hr of greening of etiolated jack bean leaves, while photosystem I activity was first observed 4 hr later.

With respect to the photosystem I activity, a correlation was observed between the appearance of a photo-oxidizable P700 molecule (the photochemical reaction center of photosystem I) and that of the photosystem I chlorophyll–protein complex (21) after 6 hr of greening. In fully green tissues the heart of the photosystem I unit is composed of 6–8 of these chlorophyll–protein molecules, each having a molecular weight of 100,000 and each containing about 12 molecules of chlorophyll *a* (21–25). One of the 6–8 chlorophyll–protein molecules contains the P700 assembly, but in all other respects it is identical to the other chlorophyll–protein molecules (22, 23). The function of these latter molecules is to feed absorbed light energy to the P700-containing entity, the site of the primary photochemical event.

As an extension of our previous investigations, we have studied the assembly of the P700-containing molecules and the light-harvesting molecules into functional photosystem I units. This has been done by examination of the composition of the photosystem I chlorophyll–protein complex isolated from leaves at different stages of greening, i.e., after 6 hr or more of illumination of etiolated leaves. In this manner it has been possible to determine whether the P700-containing chlorophyll–protein molecule is synthesized initially followed by addition of chlorophyll–protein molecules devoid of P700, or whether the 6–8 chlorophyll–protein molecules without P700 are assembled first and only later is the P700 inserted. The working hypothesis was that a preponderance of P700-containing chlorophyll–protein molecules should be present in preparations of the complex from leaves at early stages of photosystem I development, if the first alternative was correct. This would be reflected in a low chlorophyll/P700 ratio that would increase after further greening. If the second alternative was correct, however, the composition of the chlorophyll–protein preparation should be constant at all stages of greening after initiation of P700 synthesis. The results obtained support the view that the first alternative is correct.

MATERIALS AND METHODS

Plant Material. Jack beans [*Canavalia ensiformis* (L.) DC.] were germinated and grown for 7 days in total darkness at 28° with 80–85% relative humidity as described (12). On the seventh day, the etiolated seedlings were illuminated with incandescent light (General Electric 150-W cool beam flood lamps) at 750–1000 ft-cd.

Preparation of Triton Lamellar Extracts. Primary leaves were harvested at specific times during greening and chloroplast lamellar extracts were prepared. The leaves were homogenized in a Waring Blendor in 0.50 M sucrose–50 mM Tris·HCl (pH 8.0), and the brei was filtered through cheesecloth.

The cellular debris was removed by centrifugation at 10,000 × *g* for 1 min and a chloroplast lamellar pellet was collected from the supernatant by centrifugation at 20,000 × *g* for 10 min. The lamellar pellet was washed twice with 50 mM Tris·HCl (pH 8.0) to remove most of the soluble proteins; a Kontes glass homogenizer was used to suspend the lamellae in buffer, and the centrifugation was at 40,000 × *g* for 10 min. The final pellet was homogenized with a sufficient volume of 1% Triton X-100 in 50 mM Tris·HCl (pH 8.0) to yield a detergent/chlorophyll ratio of 75/1 (w/w). The Triton-solubilized lamellae were used for purification of the photosystem I chlorophyll–protein complex and for measurement of light-induced absorbance changes of P700.

Chlorophyll Determinations. A portion of the leaf samples harvested for lamellar preparations was used to determine the rate of chlorophyll accumulation during greening. Chlorophyll was measured by the procedure of Arnon (26).

Isolation of the Photosystem I Chlorophyll–protein Complex. The photosystem I chlorophyll–protein complex was isolated by the methods of J. P. Thornber, R. S. Alberte, M. Frick, and J. Shiozawa (in preparation). The Triton extract was run into a column of hydroxyapatite (27) equilibrated with 10 mM sodium phosphate (pH 7.0). A large portion of the pigmented material was eluted from the column with the 10 mM sodium phosphate buffer. The photosystem I complex was eluted subsequently with 0.2 M sodium phosphate (pH 7.0) after a brief wash of the column with 0.2 M sodium phosphate–0.1% sodium dodecyl sulfate–10 mM $MgCl_2$.

Determination of the Chlorophyll/P700 Ratio. The reversible light-induced absorbance changes due to P700 in Triton-solubilized lamellar extracts and in isolated chlorophyll–proteins were measured on an Aminco DW-2 spectrophotometer by described procedures (22). The concentration of P700 was estimated from the light-oxidized against reduced difference spectrum by use of a differential extinction coefficient for P700 at 697 nm of 100 mM^{-1} cm^{-1}. Also used was a reference wavelength of 715 nm, an isobestic point in the P700 difference spectrum. Sodium ascorbate was added to samples to fully reduce P700 in the dark, while methyl viologen was added to aid in obtaining the P700 in a fully oxidized state in the light. The chlorophyll concentration of the test sample was determined from the absorbance maximum at 676–678 nm, with an extinction coefficient of 60 mM^{-1} cm^{-1} (J. P. Thornber, R. S. Alberte, M. Frick, and J. Shiozawa, in preparation).

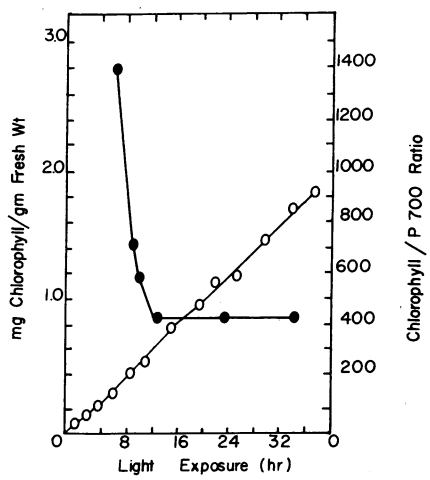

FIG. 1. Time course of accumulation of chlorophyll (○——○) and of appearance of photo-oxidizable P700 (●——●) in primary leaves of jack bean greening under high relative humidity conditions. P700 content is expressed as the chlorophyll/P700 ratio in the Triton X-100-lamellar extracts prepared from leaves after various periods of illumination.

TABLE 1. *P700 content of fractions of jack bean leaves at various stages of greening*

Light exposure (hr)	Chlorophyll/P700 ratio		Percentage of leaf chlorophyll in CPI*
	Triton extract	CPI*	
6	1400	35	2
8	720	45	6
10	600	48	8
12	395	63	16
24	400	69	17
36	380	77	22
∞	430	75	17

* CPI is the photosystem I chlorophyll–protein complex described in the text.

RESULTS

Jack bean leaves greening under the described conditions show a rapid and uniform accumulation of chlorophyll with no lag in accumulation rate (Fig. 1). These results are in agreement with those reported (12).

No P700 could be detected during the very early stages of greening in jack bean (12). Light-induced bleaching of P700 was first detected in very low concentration (chlorophyll/P700 = 1400) in leaves receiving light for 6 hr. The chlorophyll/P700 ratios in the leaf decreased during subsequent greening in a manner in agreement with that reported (12), and stabilized after about 12 hr of illumination (Fig. 1).

Analysis of the photosystem I chlorophyll–protein complex isolated from leaves greened for 6 hr gave low chlorophyll/P700 ratios (35/1). The ratio steadily increased in preparations of the complex from leaves illuminated for longer periods (Table 1), and reached a steady-state level of about 75/1 after 36 hr of greening. The same concentration of P700 was observed in preparations from leaves fully developed in the light. Table 1 includes determinations of the percentage of the total chlorophyll associated with the photosystem I chlorophyll–protein fractions at several stages of greening. It is clear that after 6 hr in the light, only a very small portion (2%) of the total chlorophyll present in the developing chloroplasts was associated with the photosystem I complex, and that during subsequent greening the amount of chlorophyll associated with this complex increases about ten times, to 22%. These results are in agreement with the observations of Kung and Thornber (24) and unpublished data of R. S. Alberte and J. P. Thornber indicating that only about 20% of the lamellar chlorophyll is associated with the photosystem I complex in higher plants.

DISCUSSION

An attempt has been made by us to answer a frequently posed question (see refs. 17, 28, 29) about one aspect of the

biogenesis of the photosynthetic membrane. Is the reaction center of a photosystem inserted into the membrane before or after the assembly of its associated light-harvesting chlorophyll? The most likely interpretation of the increase in the chlorophyll/P700 ratio of the isolated photosystem I chlorophyll–protein complex during greening (Table 1) in the present work is that the P700-containing chlorophyll–protein molecule is synthesized first and is later embellished with light-harvesting chlorophyll–protein molecules. Thus, in the case of photosystem I of the jack bean it appears that the reaction center is inserted into the membrane before the addition of much of the light-harvesting chlorophyll during chloroplast development. The only way in which our results could be interpreted to show that insertion of the reaction center is the final step in the assembly of a photosystem is to presume the photosystem I chlorophyll–protein complex, made in the early stages of greening and having a chlorophyll/P700 ratio of 35/1, receives no further additions of light-harvesting chlorophyll–protein molecules during biogenesis of other photosynthetic units. This latter interpretation seems unlikely since the chlorophyll–protein molecules probably self-assemble *in vivo* into a unit of uniform size of about 6–8 molecules—the concentration of P700 in the photosystem I chlorophyll–protein isolated from such widely different sources as seed plants and blue-green algae is the same (J. P. Thornber, R. S. Alberte, M. Frick, and J. Shiozawa, in preparation; ref. 22). Thus, it would be unlikely that synthesis of the complex during greening would be arrested at a smaller than normal size. Further support for our view on the biogenesis of photosystem I chlorophyll–protein is given by the work of Herron and Mauzerall (30), who have suggested from analysis of light-saturation curves in a greening mutant of *Chlorella* that there is a progressive addition of light-harvesting chlorophyll to rapidly formed reaction centers during greening.

The increase in the percentage of the total chlorophyll of the leaf associated with the photosystem I chlorophyll–protein during greening (Table 1) quantitates and confirms our earlier observation (12) that synthesis of the reaction center of photosystem I begins after about 6 hr of greening, and that during the next 30 hr the photosystem I chlorophyll–protein complex accounts for an increasing portion of the chlorophyll of the leaf; after 36 hr of greening the proportion of chlorophyll–protein is the same as that found in normal light-grown plants. These observations are precisely what would be expected if biosynthesis of photosystem I followed that of photosystem II.

If the generally accepted Z-scheme of photosynthesis is to function at maximum efficiency with an equal division of labor between the two photosystems, it has been maintained (see refs. 28, 29) that there should be approximately equal distribution of chlorophyll between the two photosystems. Inasmuch as the percentage of chlorophyll associated with the photosystem I chlorophyll–protein complex is only about 20% of the total, other chlorophyll molecules must be able to transfer light energy to P700 *in vivo*. It is most probable that these other chlorophyll molecules are localized in the major light-harvesting chlorophyll–protein of the chloroplast, which is a complex of chlorophylls *a* and *b* and protein previously termed the photosystem II chlorophyll–protein (23, 24, 31). Perhaps the chlorophyll *a*/*b*–protein complex performs a less-specific function than heretofore envisaged. It is a matter for future investigation to determine when and how molecules of the chlorophyll *a*/*b*–protein are added to the photosystem I unit, and whether the first photosystem I chlorophyll–protein molecule inserted into the developing thylakoids obtains its P700 in a functional form before or immediately after insertion into the membrane.

Much research during the last decade has been devoted to the isolation of P700 in a highly enriched form (32). Enrichment of seed plant and algal P700 has proved to be more difficult than for the bacterial reaction center. Thus far, the most enriched material has been isolated from spinach stroma lamellae and exhibits a chlorophyll/P700 ratio of sixteen (33), when a millimolar extinction coefficient for P700 of 60 cm^{-1} (100 cm^{-1} in this paper) is used. Owing to the small quantities present it is likely to be difficult to isolate the photosystem I chlorophyll–protein complex from tissues allowed to green for less than 6 hr. The attempt should be made, however, because that is almost certainly the best material for study of the nature of P700.

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