Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7502–7506, October 1990
Botany

Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis

(thylakoids/chloroplast acclimation/reaction center/quantum yield/light quality)

WAH SOON CHOW*†, ANASTASIOS MELIS‡, AND JAN M. ANDERSON*

*Commonwealth Scientific and Industrial Organisation, Division of Plant Industry, G.P.O. Box 1600, Canberra, Australian Capital Territory 2601, Australia; and ‡Department of Plant Biology, University of California, Berkeley, CA 94720

Communicated by Daniel I. Arnon, July 3, 1990

ABSTRACT **The efficiency of photosynthetic electron transport depends on the coordinated interaction of photosystem II (PSII) and photosystem I (PSI) in the electron-transport chain. Each photosystem contains distinct pigment–protein complexes that harvest light from different regions of the visible spectrum. The light energy is utilized in an endergonic electron-transport reaction at each photosystem. Recent evidence has shown a large variability in the PSII/PSI stoichiometry in plants grown under different environmental irradiance conditions. Results in this work are consistent with the notion of a dynamic, rather than static, thylakoid membrane in which the stoichiometry of the two photosystems is adjusted and optimized in response to different light quality conditions. Direct evidence is provided that photosystem stoichiometry adjustments in chloroplasts are a compensation strategy designed to correct unbalanced absorption of light by the two photosystems. Such adjustments allow the plant to maintain a high quantum efficiency of photosynthesis under diverse light quality conditions and constitute acclimation that confers to plants a significant evolutionary advantage over that of a fixed photosystem stoichiometry in thylakoid membranes.**

Energy transduction in photosynthesis depends on the coordinated electron turnover by two photosystems in a linear electron-transport process. Photosystem II (PSII) is involved in a light-dependent oxidation of water and reduction of plastoquinone. Electrons from plastohydroquinone reach photosystem I (PSI) via the cytochrome b_6–f complex and plastocyanin. PSI is involved in a light-dependent electron transport to ferredoxin and to $NADP^+$. Each photosystem is associated with distinct pigment–protein complexes, which absorb solar radiation and transfer excitation energy to the photochemical reaction center.

In almost every photosynthetic organism, light-harvesting pigments of PSII are different from those of PSI, thus allowing different wavelengths of light to sensitize the two photosystems unevenly. For example, wavelengths of light in the 600- to 650-nm region are absorbed preferentially by the phycobilins in cyanobacteria and red algae, or by chlorophyll b in higher plant chloroplasts. These wavelengths of light will induce a faster electron turnover at PSII than at PSI. On the other hand, wavelengths of light absorbed primarily by chlorophyll a and β-carotene will induce a faster electron turnover at PSI than at PSII (1).

The quantum yield of photosynthesis in many species from diverse light habitats is ≈0.106 ± 0.001 mol of O_2 evolved per mol of photon absorbed (2–5). This value is very close to a theoretical upper limit of 0.125 mol of O_2 evolved per mol of photon absorbed, translating to a photosynthesis efficiency of ≈85%, independent of the light climate in which plants grow. This is a remarkable feature of the photosynthetic apparatus, given the contrasting light environments in different plant ecosystems (6–8) and the fact that substantially different pigments absorb light for PSI and for PSII in the thylakoid membrane of oxygenic photosynthesis.

These findings suggested that higher plants and algae possess regulatory mechanisms that enable chloroplasts to adjust and optimize the function of the light reactions under diverse conditions. Recently, evidence in the literature suggested long-term adjustments in photosystem stoichiometry as a plant response to different light-quality conditions during growth (9, 10). Changes in photosystem stoichiometry, occurring in response to different light qualities, may be a compensation reaction in the thylakoid membrane, serving to correct uneven absorption of light by the two photosystems. However, the effect of these adjustments on the quantum yield of photosynthesis in higher plants has not been investigated before. This work provides direct evidence that adjustments of photosystem stoichiometry in chloroplasts permit the plant to retain a quantum efficiency of photosynthesis near the theoretical maximum.

MATERIALS AND METHODS

Growth of Plants. *Pisum sativum* L. cv. Greenfeast was cultivated in a growth chamber under controlled conditions (18 hr of light at 24°C/6 hr of dark at 14°C). The growth light was either incandescent illumination filtered by red Plexiglas (PSI light; ≈75 μmol of photons·m^{-2}·s^{-1}; 580–740 nm), or cool-white fluorescent illumination filtered by yellow Plexiglas (PSII light; ≈95 μmol of photons·m^{-2}·s^{-1}; 520–695 nm). The relative intensity of the two light sources was selected so that the integrated absorption of light by chloroplasts in the leaves would be about the same under PSI-light and PSII-light conditions (11). The relative spectral irradiance of each growth-light regime, measured by a spectroradiometer (SR3000A, Macam Photometrics, Livingston, Scotland), is shown in Fig. 1. Plants were harvested 20–22 days from sowing. To ensure sample uniformity, only the fourth pair of leaflets from the base was harvested and used in this study.

Assay of Thylakoid Membrane Components. Chloroplasts were isolated (12) and stored at 77 K until use. Chlorophyll concentration was determined in 80% acetone (13) using a Hitachi (Tokyo) model U-3300 spectrophotometer. The concentration of cytochrome f was determined (14) with a Hitachi 557 double-beam spectrophotometer. The concentration of PSII reaction centers was estimated from the number of 3′-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-binding sites in the thylakoid membrane (12, 15). The concentration of PSI reaction centers was estimated from the light-induced absorbance change at 703 nm (12, 16).

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Abbreviations: PSI, photosystem I; PSII, photosystem II; DCMU, 3′-(3,4-dichlorophenyl)-1,1-dimethylurea.

†To whom reprint requests should be addressed.

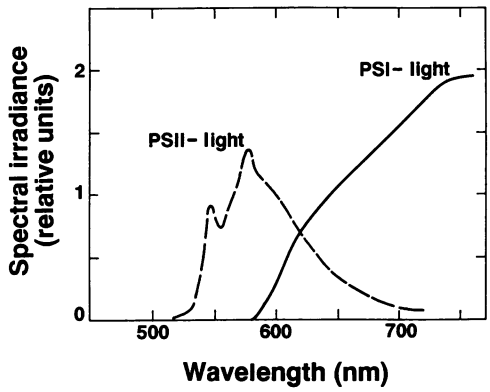

FIG. 1. Spectral distribution of irradiance in each growth light environment, designed to favor excitation of one photosystem over the other.

Leaf Photosynthesis Measurements. Rates of O_2 evolution at 25°C and ≈1% CO_2/99% air were measured with a Hansatech (Kings Lynn, U.K.) leaf disc oxygen electrode (17). Actinic light for these measurements was provided by a quartz halogen light bulb. The white light was filtered to give a predominantly PSI or PSII irradiance. PSI irradiance was obtained by passing the white actinic light through red Plexiglas (Rohm and Haas, no. 2423). PSII irradiance was obtained by a combination of yellow Plexiglas (Rohm and Haas, no. 2208) and a long-wavelength cut-off filter [Ealing (Holliston, MA), 35-5453 VIQ 5–8]. The predominantly PSI or PSII irradiance for the measurement of O_2 evolution was similar to the respective PSI and PSII light conditions used for plant growth (Fig. 1). The intensity of the actinic light was varied by a combination of Balzers neutral density filters and was measured directly at the position of a leaf disc with a LiCor quantum sensor (LI 188B; Lambda Instruments, Lincoln, NE).

The fraction of incident PSII irradiance absorbed by a leaf at the oxygen electrode (absorptance) was determined by measuring the transmittance and reflectance of the leaf with an integration sphere attached to the LiCor quantum sensor. To estimate the absorptance of the PSI irradiance by a leaf, a transmittance spectrum was obtained by placing the leaf against the photomultiplier tube of a Hitachi 557 double-beam spectrophotometer. The absorptance of the leaf was calculated as (1 − transmittance), assuming a zero absorptance at 800 nm. Leaves grown under PSI light absorbed 76% of the incident PSI irradiance and 79% of the incident PSII irradiance. Leaves grown under PSII light absorbed 82% of the PSII irradiance and 84% of the PSI irradiance.

RESULTS

Concentration of Pigments and of Electron-Transport Components in Thylakoid Membranes. Illumination conditions for the growth of plants in the present study were chosen to provide a light quality environment that favors excitation of one photosystem over the other. Yellow light (PSII light) was used to preferentially excite PSII, while red light (PSI light) preferentially excites PSI (Fig. 1). To define the biochemical responses of the photosynthetic apparatus to the resulting long-term imbalance in light absorption by the two photosystems, we measured the chlorophyll content of leaves, the chlorophyll a/b ratio, and the concentrations of cytochrome *f*, PSII, and of PSI in the thylakoid membrane.

Pea plants acclimated to PSI light conditions had ≈413 μmol of chlorophyll per m^2 of leaf area, compared with 555 μmol of chlorophyll per m^2 in the leaves of PSII light-grown plants (Table 1). The greater chlorophyll content per leaf area in PSII than in PSI light-grown plants could not be attributed to the differential rate of light absorption by the two photosystems (18). It probably reflects a differential activation of phytochrome and/or of a blue light receptor that has resulted in dissimilar leaf thickness and dissimilar chloroplast density in the cells of the two pea samples. Moreover, the chlorophyll a/b ratio of thylakoids isolated from plants grown in PSI light (PSI light thylakoids) was lower compared with that of PSII light thylakoids. These results indicated differences in the pigment composition of the leaves and/or in the relative amount of PSII and PSI units in the thylakoid membrane of the two samples (19–21). However, on a chlorophyll basis, the concentration of cytochrome *f*, and therefore the cytochrome b_6-*f* complex, was essentially the same in PSI and PSII light thylakoids (Table 1).

Quantitation of reaction centers was obtained from the number of DCMU-binding sites (PSII measurement) and from the amount of photooxidizable P700 (PSI measurement) in isolated thylakoid membranes (16, 22). Table 1 shows that PSI light thylakoids had a greater number of DCMU-binding sites (greater PSII reaction center concentration) per unit of chlorophyll, compared with PSII light thylakoids. In contrast to the results from the PSII assay, the concentration of photooxidizable P700 (PSI) was greater in PSII light thylakoids than in PSI light thylakoids.

Table 1 summarizes the chlorophyll and photosystem quantitation results of three independent experiments. There was a significant difference in the relative concentration of the two photosystems between the two kinds of samples. Compared with sunlight-grown peas (PSII/PSI = 1.8:1.0), PSI light thylakoids had a higher PSII/PSI reaction center stoichiometry (2.5:1.0), whereas PSII light thylakoids had a lower ratio (1.1:1.0). These results are consistent with earlier findings (11, 19–22).

The acclimation of the thylakoid membrane to PSI and PSII light did not entail significant changes in the size and composition of the functional chlorophyll antenna size of the two photosystems. In agreement with earlier results (21), PSII light conditions elicited a 10% larger PSII antenna size and a 5% larger PSI antenna size, compared with those of PSI light conditions. These are small and parallel changes compared with the large antiparallel adjustments in photosystem stoichiometry. Thus, changes in the light-harvesting antenna are not expected to influence the quantum yield of photosynthesis under the two experimental light regimes.

Leaf Photosynthesis Characteristics. The adjustment of photosystem stoichiometry in pea thylakoids raised the question of the effect these changes might have on the quantum yield of photosynthesis. To address this question, we measured the light-saturation curve of photosynthesis in leaves from the two pea cultures. The rate of photosynthesis *in vivo*

Table 1. Quantitation of pea thylakoid components

	Chlorophyll content	Chlorophyll a/b	Cytochrome *f*	DCMU-binding sites	P700	PSII/PSI
PSI light thylakoids	413 ± 8	1.97 ± 0.02	1.17 ± 0.08	2.67 ± 0.07	1.05 ± 0.09	2.5
PSII light thylakoids	555 ± 17	2.24 ± 0.01	1.04 ± 0.07	1.97 ± 0.05	1.73 ± 0.08	1.1

Chlorophyll (a + b) content is given in μmol·m^{-2} of leaf area. Chlorophyll a/b (mol/mol) ratios and concentrations (mmol per mol of chlorophyll) of cytochrome *f*, DCMU-binding sites (PSII), and P700 (PSI) are shown for pea thylakoids grown under predominantly PSI or PSII light. Each value is the mean ± SE of three separate experiments.

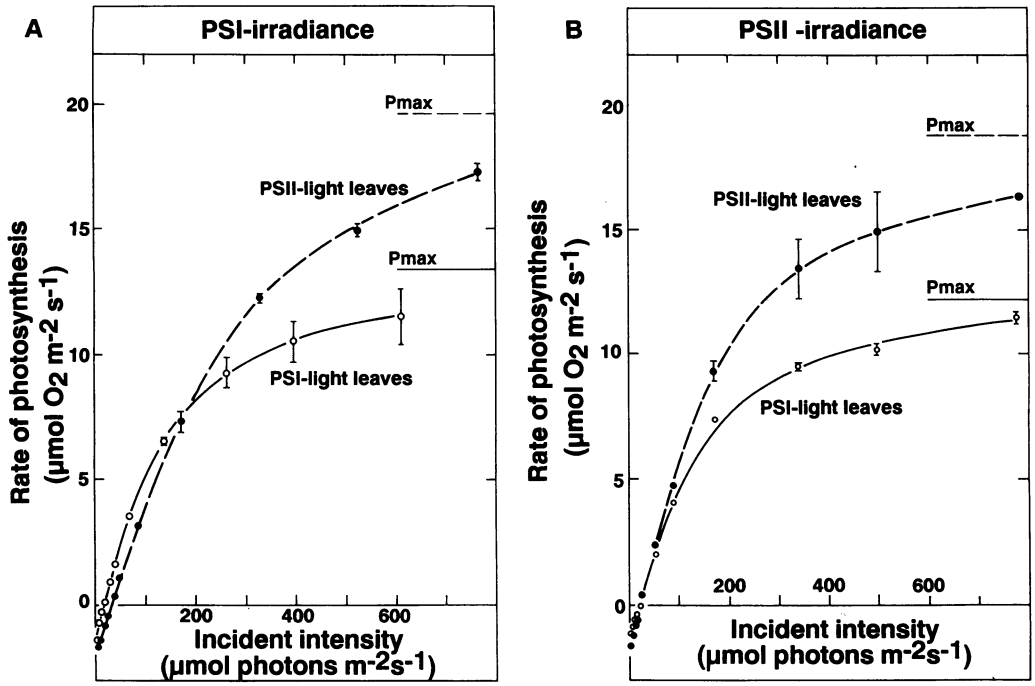

FIG. 2. Light-saturation curves of photosynthesis in pea plants grown under predominantly PSI or PSII light and probed by either PSI irradiance (*A*) or PSII irradiance (*B*). Horizontal lines show the light-saturated rates of photosynthesis (P_{max}) elicited by strong white light (≈1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The mean values of photosynthetic rates ± SE are shown for three replicates in *A* and for five replicates in *B*.

was measured from the rate of O_2 evolution per unit leaf area separately under predominantly PSI irradiance (Fig. 2*A*) or PSII irradiance (Fig. 2*B*). In the dark, the rate of respiration was slightly greater for PSII light leaves. Under saturating light, the PSII light leaves displayed higher capacities for photosynthetic O_2 evolution per unit leaf area. The higher capacity for respiration and photosynthesis per unit leaf area in PSII light leaves correlates with the higher chlorophyll content per unit leaf area in these samples (Table 1).

The results of Fig. 2 also show different shapes of the light-saturation curves of photosynthesis for PSI and PSII light-grown leaves when probed by PSI irradiance (Fig. 2*A*) or by PSII irradiance (Fig. 2*B*). Although the rate of photosynthesis is plotted as a function of incident intensity on the leaves, the initial slopes of the light-response curves suggested a dissimilar dependence of the quantum yield of photosynthesis in the two samples on the quality of the actinic light. To address this question rigorously, the absolute quantum yield of photosynthesis was measured in PSI and PSII light-grown leaves by using predominantly PSI or PSII irradiance conditions. Fig. 3*A* shows the initial slopes of the light-response curves of PSI and PSII light-grown pea leaves as a function of absorbed PSI irradiance. The slope of the straight lines defined the quantum yield of photosynthesis for the two samples. We calculated that PSI light-grown leaves had a quantum yield of 0.108 while PSII light-grown leaves had a lower quantum yield of 0.091 (Table 2). Under PSII irradiance (Fig. 3*B*), the quantum yield of PSI light-grown leaves was 0.080, whereas that of PSII light-grown leaves was 0.097 (Table 2). By comparison, the mean value of the quantum yield (measured in white light from a quartz halogen lamp) for 37 C_3 pathway species, grown under a variety of physiological and nonstressful conditions, was 0.106 ± 0.001 (3–5). The results of Table 2 show that, under PSI irradiance, PSI light-grown leaves have a 19% better quantum yield of photosynthesis than PSII light-grown leaves. Conversely

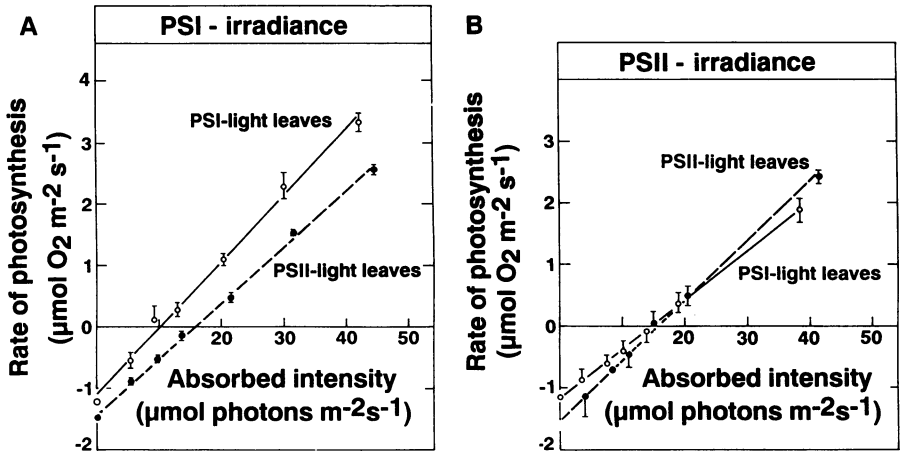

FIG. 3. The initial linear portion of the light-saturation curve of photosynthesis as a function of absorbed intensity in PSI and PSII light-grown leaves. Leaves were probed with PSI irradiance (*A*) or PSII irradiance (*B*). The slope of the straight lines defined the quantum yield of photosynthesis. The mean values of photosynthetic rates ± SE are shown for three replicates in *A* and for five replicates in *B*.

Table 2. Quantum yield of photosynthesis in pea leaves

	PSI irradiance	PSII irradiance
PSI light leaves	0.108	0.080
PSII light leaves	0.091	0.097
Difference, %	+19	−21

The quantum yields are given as mol of O_2 evolved per mol of photons absorbed. Pea leaves were grown under preferentially PSI light or PSII light conditions. Quantum yield measurements were taken with irradiance sensitizing primarily PSI or PSII.

under PSII irradiance, PSII light-grown leaves have a 21% better quantum yield than PSI light-grown leaves.

DISCUSSION

In 1960, the organization of the electron-transport chain in chloroplasts was formulated in the so-called Z-scheme (23, 24). Implicit in the original hypothesis of the Z-scheme was the assumption that optimal electron flow in the thylakoid membrane, and a high quantum yield of photosynthesis, would occur only if the two photosystems existed in equal stoichiometric amounts. This assumption of an obligatory 1:1 stoichiometric ratio between PSII and PSI was not correctly tested for about 20 years. The advent of sensitive spectrophotometric methods for the quantitation of integral components within each photosystem offered the opportunity in 1980 to address the question of photosystem stoichiometry in oxygenic photosynthesis (25). The assumption of a PSII/PSI = 1:1 ratio was not confirmed. Results indicated a large variability in the ratio of PSII and PSI reaction centers (from 0.43 in cyanobacteria to 3.3 in chlorophyll b-deficient mutant and developing chloroplasts) (25). Moreover, research over the last 10 years in several laboratories (9, 10) revealed dynamic features in the composition and function of thylakoid membranes and strongly suggested that oxygen-evolving plants are able to adjust and optimize the stoichiometry of the electron-transport complexes in response to irradiance change.

The present study provides evidence that changes in photosystem stoichiometry optimize electron transport in the thylakoid membrane and help maintain a high quantum yield of photosynthesis. This was particularly evident when pea plants were acclimated to predominantly PSI light conditions, and the PSII/PSI ratio increased to 2.5, compared with 1.8 in control plants. When probed with PSI irradiance—i.e., the light these plants were grown under—the PSI light-grown leaves had a quantum yield of photosynthesis equal to 0.108 mol of O_2 evolved per mol of photon absorbed, compared with the quantum yield of 0.091 for the PSII light-grown leaves. This finding suggests a cause and effect relationship between the elevated PSII/PSI ratio and the higher quantum efficiency of photosynthesis in PSI light-grown pea plants. The converse was true for pea plants that were acclimated to predominantly PSII light conditions (Table 2).

Thus, it is evident that adjustments of photosystem stoichiometry optimize the PSII/PSI ratio in the thylakoid membrane and help the plant to retain a high quantum efficiency of photosynthesis. This conclusion underscores the dynamic nature of thylakoid membrane composition and function in higher plant chloroplasts and has important implications for plant growth and productivity under physiological conditions. In the terrestrial environment, where most higher plants live, there are pronounced gradients in light quality within a single leaf (8) and within the canopy of a single tree or within the canopy of a forest (6). Similarly, marked variation in the light quality occurs within the aquatic environment (7). Most of these gradients in light quality would result in preferential absorption of light by one photosystem over the other, thus upsetting the balance of light utilization by the two photoreactions. If left uncorrected, they would tend to lower the efficiency of light utilization by higher plants in terrestrial environments (Table 2), or by algae in their aquatic environment (26, 27), thereby affecting adversely plant growth and productivity.

It is clear that the mechanism for photosystem stoichiometry adjustment is well preserved in all oxygen-evolving organisms from cyanobacteria to higher plants (9). The adjustment and optimization of the photosystem stoichiometry in thylakoid membranes enable photosynthetic cells to operate efficiently under a broad variety of light-limiting conditions. This is important since most higher plants and algae grow in habitats of limiting light conditions. Furthermore, it is increasingly evident that most of the canopy in crop plants operate at light levels below those required to saturate the rate of photosynthesis (28). From the evolutionary point of view, chloroplasts possessing such an adaptation mechanism will enjoy a significant selective advantage over others with a fixed photosystem stoichiometry in their thylakoid membranes.

Very little is known about the molecular and biochemical basis of the feedback control mechanism for the regulation of photosystem stoichiometry (9). Measurements on the rate of photosystem stoichiometry adjustment, both in higher plant chloroplasts (29) and in cyanobacteria (30), have shown a half-time of change of ≈20 hr. This suggested the involvement of both biosynthetic and degradative reactions in the process of thylakoid membrane acclimation. Preliminary evidence has suggested control via regulation of the steady-state level of mRNA coding for photosystem components (11, 31) and via regulation by protein phosphorylation in photosynthetic cells (32). Clearly, more research is needed to unravel further details of this important phenomenon.

This research was supported in part by a Pacific Rim/University of California Faculty Exchange Award and National Science Foundation Grant DMB-9003928 to A.M. and an Australian National Research Fellowship to J.M.A. and W.S.C. We thank Ms. Steph Hossack-Smith for her excellent technical assistance.

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