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Stoichiometry of system I and system II reaction centers and of plastoquinone in different photosynthetic membranes

(photoreaction/electron transport/spectrophotometry/chloroplast function/P700-chlorophyll)

ANASTASIOS MELIS AND JEANETTE S. BROWN

Carnegie Institution of Washington, Department of Plant Biology, 290 Panama Street, Stanford, California 94305

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ABSTRACT **The concentrations of photochemical centers and of plastoquinone were measured in several kinds of photosynthetic membranes by optical difference spectroscopy. Photosystem I reaction centers were measured from the light-induced absorbance change at 700 nm (oxidation of the primary electron donor, P700). Photosystem II reaction centers were estimated from the light-induced absorbance change at 325 nm (reduction of the primary electron acceptor, Q). Spinach chloroplasts and membrane fractions obtained by French press treatment, mature and developing pea chloroplasts, and blue-green algal membranes were investigated. No loss of primary photochemical activity occurred during fractionation of the chloroplasts. The results indicated a large variability in the ratio of system II to system I reaction centers (from 0.43 to 3.3) in different photosynthetic membranes. Oxygen-evolving plants may change the ratio of their photosystems in response to environmental light conditions. The amount of photoreducible plastoquinone was also measured at 263 nm. In spinach chloroplasts, seven to eight plastoquinone molecules were found per reaction center of system II. Most of the plastoquinone pool was associated with the grana. However, the ratio of chemically determined plastoquinone to chlorophyll was similar in the grana and stroma thylakoids.**

Green plant photosynthesis is considered to take place by way of two photoreactions. The trapping of excitation energy by the reaction center of photosystem II (PSII) causes the reduction of the primary electron acceptor, Q, a specialized plastoquinone (PQ) molecule that serves as a one-electron-acceptor (1–5). Excitation of the photosystem I (PSI) reaction center causes the oxidation of P700, a special type of chlorophyll (Chl) (6). The stoichiometric relationship between the two photosystems is generally assumed to be close to unity, although such an assumption has not been thoroughly investigated. On the contrary, there are recent reports in the literature suggesting that at least in blue-green algae (7, 8) and in some tobacco mutants (9) the ratio of PSII to PSI reaction centers deviates significantly from unity. Haehnel (10) reported a functional differentiation within PSI in spinach chloroplasts and a ratio slightly deviating from unity.

In the experiments reported here we determined the number of PSII and PSI reaction centers in different photosynthetic membranes. A spectrophotometric method allowed us to look directly at the reaction center complexes of PSI and PSII by monitoring the concentrations of $P700^+$ and Q^-, respectively. We found wide variations in the ratio of PSII to PSI reaction centers (from 0.43 to 3.3) depending on the plant material, growth conditions, and the kind of chloroplast membrane examined (grana or stroma lamellae). The physiological significance of these variations is discussed. In addition, the amount of photoreducible and total PQ in spinach chloroplasts, grana, and stroma lamellae was measured.

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MATERIALS AND METHODS

Chloroplasts were prepared from leaves of spinach (*Spinacea oleracea* Linnaeus) obtained from a local market, by following the procedure of Sane *et al.* (11). The chloroplast pellet was resuspended in 150 mM KCl and 50 mM *N*-[tris(hydroxymethyl)methyl]glycine (Tricine), pH 7.8, and passed once through an Aminco French pressure cell at 400 kg cm^{-2} (39 MPa) to disrupt the photosynthetic lamellae. The resulting mixture of membrane fragments was centrifuged at 1000 × *g* for 10 min to remove unbroken chloroplasts. Subsequent centrifugations at 10,000 × *g* for 30 min, 40,000 × *g* for 30 min, and 160,000 × *g* for 60 min resulted, respectively, in the sedimentation of heavy (grana lamellae, 10,000), intermediate (40,000), and light (stroma lamellae plus end membranes of grana, 160,000) fractions (11) which were resuspended in the same medium. The Chl concentrations and Chl *a*/*b* ratios were determined according to Mackinney (12).

Pea chloroplasts were isolated from *Pisum sativum* Linnaeus plants cultivated in the laboratory. Seven-day-old etiolated pea seedlings were illuminated by cool-white fluorescent lamps at 17 W m^{-2} either continuously or intermittently (3 hr light, 21 hr dark). Chloroplasts were isolated from the pea leaves on the seventh day of greening.

Photosynthetic membranes from the thermophilic blue-green alga *Synechococcus lividus* (13) grown at 55°C were isolated by treatment with lysozyme (14) followed by rupture in a French press and centrifugation at 40,000 × *g* for 1 hr.

The amplitudes of the light-induced absorbance changes at 700, 325, and 263 nm were measured with a split-beam spectrophotometer that was constructed in our laboratory and has a resolution of less than 10^{-4} ΔA in the ultraviolet. The reaction mixtures contained 250 μM Chl in 5 mM $MgCl_2$/10 mM KCl/50 mM Tricine, pH 7.8. For P700 measurements, 12 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 1.2 mM sodium ascorbate, 0.5 μM dichloroindophenol, and 60 μM methylviologen were included; for Q measurements, 12 μM DCMU and 1.5 mM potassium ferricyanide; and for PQ measurements, 10 μM gramicidin. In the latter case the absorbance change at 263 nm was corrected for any change at 285 nm (the isosbestic point for the PQ change). The optical pathlength of the cuvette for the measuring beam was 1.4 mm and for the actinic beam (saturating light transmitted by CS 4-96 and CS 3-68 Corning filters) it was 1.0 mm.

It was important to correct the light-induced absorbance changes for the flattening effect particles may have on absorption bands (15). This was routinely accomplished according to the method given by Pulles *et al.* (16). For some of the measurements, the correction for flattening was also estimated

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone; PS, photosystem; P700, primary electron donor of PSI; Q, primary electron acceptor of PSII; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine.

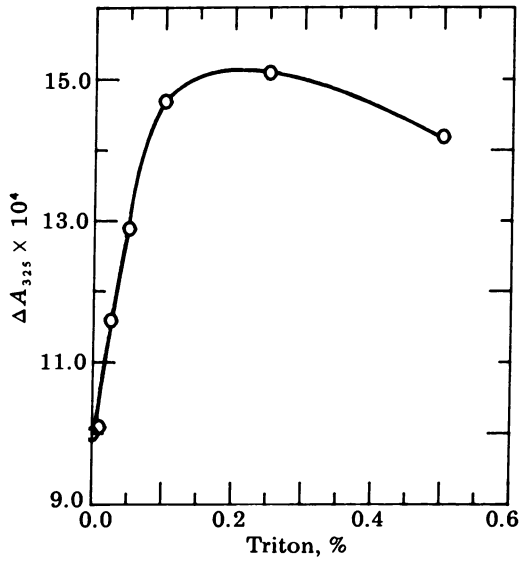

FIG. 1. Effect of Triton X-100 concentration, measured within 5 min, on the PSII absorbance change at 325 nm of spinach chloroplasts. The signal increase is caused by solubilization of the chloroplast membranes and the concomitant elimination of particle flattening of absorption (15, 16).

by the following method: Fig. 1 shows the effect of Triton X-100 concentration on the amplitude of the light-induced absorbance change at 325 nm attributed to the reduction of Q. Low concentrations of Triton dispersed the chloroplast membranes, thus eliminating flattening and light-scattering without having a significant effect on the photoreduction of Q. Higher concentrations (>0.25%) caused inactivation of the photoreaction. In the same chloroplast preparation the differential particle flattening determined by the method of Pulles *et al.* (16) was 1.55, in good agreement with the effect of Triton on the signal amplitude at 325 nm.

Our experimental conditions either suppressed or fully eliminated absorbance changes caused by electron transfer components other than those investigated. In the ultraviolet, we have subtracted the contribution of the P700 absorbance change to that of Q and the contribution of the Q absorbance change to that of PQ.

The concentrations of P700, Q, and PQ were calculated from the amplitude of their respective light-induced absorbance change and differential extinction coefficient [$\Delta\epsilon$ = 64 mM^{-1} cm^{-1} at 700 nm for P700 (17), 11 mM^{-1} cm^{-1} at 325 nm for Q (3–4), 13 mM^{-1} cm^{-1} at 263 nm for PQ (18)]. The random error involved in our photochemical determinations was estimated with several samples of the same chloroplast preparation and was found to be approximately ±15% for measurements of Q and ±5% for P700 and PQ.

The chemical determination of PQ A in each of the chloroplast fractions was performed according to Bishop and Wong (19). After PQ had been isolated by thin-layer chromatography, its concentration was measured from the oxidized-minus-reduced absorbance difference at 255 nm, using $\Delta\epsilon$ = 15 mM^{-1} cm^{-1} (20).

RESULTS

To verify the identity of the light-induced absorbance changes at 263, 325, and 700 nm under our experimental conditions, we measured the light-minus-dark difference spectrum of each change. Fig. 2*A* shows the absorption difference spectrum caused by the photoreduction of plastoquinone to plastohydroquinone (2). Fig. 2*B* shows the difference spectrum of photoreduced Q, which indicates the transition of the primary electron acceptor of PSII to its reduced semiquinone anion form, Q^- (2–5). Fig. 2*C* identifies the well-known light-induced oxidation of the PSI reaction center, P700 (6, 17).

The average distributions of Chl, P700, Q, and PQ in four different grana and stroma membrane preparations from spinach are shown in Table 1. The data in Table 1 have been normalized to correspond to 100 μmol of Chl (*a* + *b*) in the chloroplasts. The first line shows the amount of Chl (% of recovery) in the three fractions (10,000, 40,000, and 160,000). In different preparations, we were able to collect 3–11% of the total Chl in the light (160,000) fraction, depending on the extent of breakage by the French press. The second line compares the average Chl *a*/*b* ratio of chloroplasts with those values found in the fractions. As expected, the 10,000 (grana) fraction was enriched in Chl *b* (Chl *a*/*b* ranging between 2.1 and 2.4), whereas the 160,000 (stroma and end grana thylakoids) fraction was enriched in Chl *a* (Chl *a*/*b* between 3.2 and 5.0).

The last column in Table 1 shows the total amount (sum) of P700, Q, and PQ recovered in the three fractions. A comparison of the total P700 measured in the fractions with the value for the chloroplasts or homogenate verifies that no loss of primary photochemical activity occurred during the fractionation process. Likewise, the amount of Q recovered in the three fractions is, within experimental error, similar to the Q content of the chloroplasts or homogenate, again indicating that no loss of primary PSII activity occurred during fractionation.

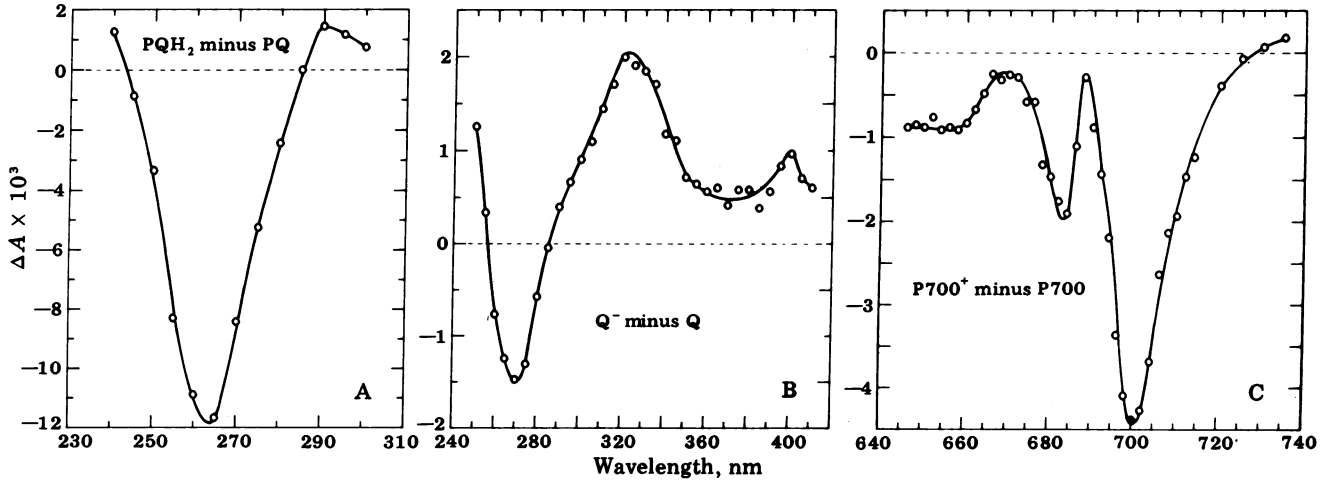

FIG. 2. Light-induced difference spectra of plastohydroquinone-minus-plastoquinone (*A*), the reduced-minus-oxidized forms of the primary electron acceptor (Q) of PSII (*B*), and the oxidized-minus-reduced forms of P700 (*C*).

Table 1. Distribution of PSI (P700) and PSII (Q) reaction center complexes and PQ in chloroplast membrane fractions

	Chloroplasts	French press homogenate	Fractions 10,000	Fractions 40,000	Fractions 160,000	Sum of fractions
Chl $a + b$, μmol	100	100	70.5	22.5	7	100
Chl a/b	2.71	2.66	2.34	2.99	3.68	
P700, μmol	0.190	0.179	0.102	0.052	0.026	0.180
Q, μmol	0.38	0.39	0.35	0.07	0.01	0.43
Chl/P700	526	558	691	433	269	
Chl/Q	263	256	201	321	700	
PQ, μmol (photochemical)	3.15	2.02	0.88	0.02	0.0	0.90
PQ, μmol (chemical)	6.00	6.00	3.2	0.19	0.12	3.51

Table 1 also shows the calculated average ratios of Chl/P700 and Chl/Q in the different preparations. In agreement with earlier results (11, 21–24) we found the heavy (10,000) fraction considerably enriched in PSII (Q) and the light (160,000) fraction enriched in PSI (P700). It must be noted that, in chloroplast preparations from spinach purchased at different times of the year, we found a constancy of Chl/P700. However, the Chl/Q ratio was markedly lower during the winter months (a ratio change from 360 in the summer to 208 in the winter). This observation raises the question whether environmental conditions regulate the ratio of the two photoreactions. Nevertheless, regardless of the initial Q and P700 content, we were always able to obtain a separation and enrichment for PSII and PSI reaction centers in grana and stroma membranes, respectively.

Assuming that for each PSII reaction center there is one primary electron acceptor molecule, Q, one can calculate the ratio of PSII to PSI reaction centers (Q/P700). Fig. 3 shows the PSII/PSI ratio as a function of the Chl a/b ratio for intact chloroplasts and for the 10,000, 40,000, and 160,000 fractions (all isolated from "summer" spinach). In the grana (10,000) fraction, the PSII reaction centers outnumber those of PSI by at least two to one. With increasing Chl a/b, there is a gradual transition to a situation in which the PSI centers are predominant. Thus in the stroma and end thylakoids of the grana (160,000) we have found as many as four PSI centers for every PSII center. These results are important in terms of chloroplast structure and function because they clearly indicate that a one-to-one stoichiometry between PSII and PSI centers is the exception rather than the rule in any membrane group of chloroplast thylakoids from spinach.

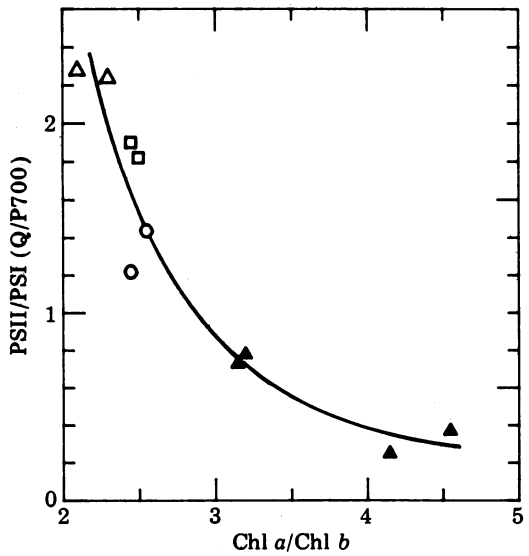

FIG. 3. Ratio of PSII to PSI reaction centers (Q/P700) as a function of the Chl a/b ratio in different subchloroplast fragments (△, grana; □, mixed; ▲, stroma) and chloroplasts (○) from summer spinach.

In freshly isolated chloroplasts the amount of photoreducible PQ (3.15 μmol) was about half of the total determined chemically (6.00 μmol) but nearly the same as the amount of chemically determined, membrane-bound PQ in the grana membranes (3.2 μmol) (Table 1). It was found that the amount of photoreducible PQ was decreased after French press treatment and differential centrifugation, suggesting detachment of PQ molecules from the electron transport chain (compare in Table 1 the photochemically and chemically determined PQ content). Because the PQ values obtained chemically were from different preparations, they cannot be directly compared with the Chl concentrations of the three fractions in Table 1, but in fact the ratio of total Chl to PQ (chemical) was approximately the same in each fraction (20 to 30).

The quantitative measurements of PSII and PSI reaction center concentration were extended to developing chloroplasts of peas and photosynthetic membranes of a blue-green alga. Under our greening conditions, the development of the pea chloroplasts is greatly retarded, apparently because of the light-limited rate of Chl biosynthesis (25, 26). Compared to control chloroplasts, our developing pea plastids had higher Chl a/b ratios (Table 2). In addition, the amount of Chl per reaction center of PSII, PSI, or both was lower. Because the membranes of developing chloroplasts are predominately exposed to the stroma and in view of our results with the stroma membranes of spinach, one might expect such chloroplasts to be enriched in PSI centers; but the opposite was observed. Table 2 shows that in the developing chloroplasts the stoichiometric ratio of PSII to PSI reaction centers was 3.3; i.e., profoundly altered in favor of PSII. However, the same ratio measured in mature pea chloroplasts was only 0.77. A quantitatively similar observation has been reported earlier (9) from tobacco mutant chloroplasts that, like developing chloroplasts, had not synthesized the Chl *ab* light-harvesting complex.

Finally, photosynthetic membranes isolated from a blue-green alga were enriched in P700. The stoichiometric ratio of PSII to PSI reaction centers was 0.43, indicating the existence of at least two PSI centers for each PSII center in these organisms (Table 2).

Table 2. Stoichiometry of PSI (P700) and PSII (Q) in mature and developing chloroplasts and a blue-green alga

	Chloroplasts			Blue-green alga
	Spinach, "summer"	Pea Mature	Pea Developing	
Chl a/Chl b	2.56	2.50	7.9	—
Chl/P700	508	513	338	133
Chl/Q	356	669	102	308
PSII/PSI	1.43	0.77	3.3	0.43

DISCUSSION

The results presented in this work clearly show that the stoichiometric ratio of PSII and PSI reaction centers can vary over a wide range (from 0.43 to 3.3) in photosynthetic membranes from different species as well as between membrane preparations from the same species. Our determinations were performed by optical spectroscopy in the red and ultraviolet spectral regions, where integral components of the PSI and PSII reaction centers, respectively, show characteristic light-minus-dark absorbance changes. Although the extinction coefficient of P700 is generally accepted (17), a somewhat greater uncertainty exists for the value of the coefficient of Q, reported so far only by van Gorkom (3, 4). Over- or underestimation of this value would cause a systematic error in our determination of PSII reaction center concentration. Because Fujita (7) and Kawamura *et al.* (8) reported similar values for the ratio of the two photoreactions in blue-green algae from O_2 flash yield measurements, such a systematic error is probably not significant. The method employed in this work for the quantitative determination of PSII and PSI reaction centers offers the advantage of looking directly at the reaction center complex of each photosystem.

From earlier work on chloroplast fractionation (11, 21–24), it has been qualitatively determined that membranes of the grana are enriched in PSII activity, whereas stroma membranes show primarily PSI activity. In this work we present a quantitative determination of the concentration of PSII and PSI reaction centers in the two structurally distinct chloroplast membranes. In the grana of spinach chloroplasts we have determined a ratio of PSII to PSI centers higher than 2, whereas in stroma membranes the ratio drops to a value as low as 0.3. These results are important for our understanding of the organization of electron transfer between the two photosystems. Assuming there are no electron transfer interactions between PSII units in the grana and PSI units in the stroma, one can accommodate the electron transfer pattern in the grana of spinach chloroplasts by a scheme according to which a number of PSII centers are connected through a common large PQ pool to relatively fewer PSI centers (10). We estimated the ratio of PSII to PQ to PSI in the grana to be approximately 5:(35–40):2. Apparently, these values can vary in different plants or in the same plant grown under different conditions. Our results imply that linear electron transfer from H_2O to $NADP^+$ in the grana is accomplished by the cooperation of several, but not stoichiometrically equal, PSII and PSI units associated with the same electron transfer chain (10). The rates of electron flow through each photoreaction center will be determined by the number of antenna pigment molecules servicing each center or, in other words, their absorption cross section.

Considering the situation in the stroma membranes, at this point we cannot state with certainty whether the observed low content in PSII is real or represents contamination from the grana. There are reports in the literature indicating the existence of PSII directly exposed to the stroma medium (9, 22, 26–28). Possible functions for PSI located in the stroma membranes have been proposed elsewhere (29).

One interesting result from our work is the unexpected difference in the Q/P700 ratio between developing and mature pea chloroplasts. In the former there is an excess of PSII reaction centers, whereas the ratio is closer to unity in the latter (Table 2). A similar difference was encountered earlier between chloroplasts from tobacco mutants and wild type (9). At present it is difficult to point to any single guiding principle with which to understand the significance in the change of the photochemical reaction center ratio. Both in the developing pea (25, 26) and in tobacco mutant chloroplasts, a significant part of the Chl *b*-containing light-harvesting complex was lacking, and their ultrastructures showed predominantly stroma-exposed thylakoids. Therefore, one might speculate that the higher PSII to PSI reaction center ratio in these chloroplasts is related to the pigment composition, structural alterations, or both. Because these chloroplasts lack the predominantly PSII antenna Chl, they may synthesize a greater number of PSII reaction centers (having a smaller effective absorption cross section) in order to compensate for the slower turnover of electrons by these units. Alternatively, PSII and PSI units may reside largely in structurally distinct thylakoid membranes or membrane areas. Thus the situation with developing peas and tobacco mutants may reflect the order by which particular chloroplast compartments are synthesized under retarded growth conditions.

The question of the photochemical reaction center ratio in blue-green algae and higher plant chloroplasts along with the thylakoid differentiation into grana and stroma membranes in the latter should be further examined in relation to the photosynthetic electron transport capacity and enzymic content in each case. An evaluation of such information may reveal the importance of having varying ratios of the two photoreactions and, in addition, may increase our understanding of chloroplast structural and functional aspects.

The PQ pool intermediate between the two photosystems is probably of key importance in realizing linear electron transfer. Our results showed (Table 1) that approximately 50% of the quinone molecules in a chloroplast were not membrane bound but were readily released into the medium by French press treatment. A small amount of the membrane-bound PQ was located in the stroma membranes. The functional role of these molecules is not clear at present, and more work in this direction is needed.

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1. Duysens, L. N. M. & Sweers, H. E. (1963) in *Studies on Microalgae and Photosynthetic Bacteria* (University of Tokyo Press, Tokyo), pp. 353–372.
2. Stiehl, H. H. & Witt, H. T. (1968) *Z. Naturforsch. Teil B* **23**, 220–224.
3. van Gorkom, H. J. (1974) *Biochim. Biophys. Acta* **347**, 439–442.
4. van Gorkom, H. J. (1976) Dissertation (Rijksuniversiteit, Leiden, The Netherlands).
5. Melis, A. & Duysens, L. N. M. (1979) *Photochem. Photobiol.* **29**, 373–382.
6. Kok, B. & Hoch, G. (1961) in *Light and Life*, eds. McElroy, W. D. & Glass, B. (Johns Hopkins Press, Baltimore, MD), pp. 397–416.
7. Fujita, Y. (1976) *Plant Cell Physiol.* **17**, 187–191.
8. Kawamura, M., Mimuro, M. & Fujita, Y. (1979) *Plant Cell Physiol.* **20**, 697–705.
9. Melis, A. & Thielen, A. P. G. M. (1980) *Biochim. Biophys. Acta* **589**, 275–286.
10. Haehnel, W. (1976) *Biochim. Biophys. Acta* **423**, 499–509.
11. Sane, P. V., Goodchild, D. F. & Park, R. B. (1970) *Biochim. Biophys. Acta* **216**, 162–178.
12. Mackinney, G. (1941) *J. Biol. Chem.* **140**, 315–322.
13. Fork, D. C. & Murata, N. (1977) *Carnegie Inst. Washington Yearb.* **76**, 222–226.
14. Sigalat, C. & deKouchkovsky, Y. (1974) in *Proceedings of the Third International Congress on Photosynthesis*, ed. Avron, M. (Elsevier, Amsterdam), pp. 621–627.
15. Duysens, L. N. M., (1956) *Biochim. Biophys. Acta* **19**, 1–12.
16. Pulles, M. P. J., van Gorkom, H. J. & Verschoor, G. A. M. (1976) *Biochim. Biophys. Acta* **440**, 98–106.

17. Hiyama, T. & Ke, B. (1972) *Biochim. Biophys. Acta* **267,** 160–171.
18. Amesz, J. (1973) *Biochim. Biophys. Acta* **301,** 35–51.
19. Bishop, N. I. & Wong, J. (1974) *Ber. Deutsch. Bot. Ges.* **87,** 359–371.
20. Barr, R. & Crane, F. L. (1971) *Methods Enzymol.* **23,** 379–408.
21. Anderson, J. M. & Boardman, N. K. (1966) *Biochim. Biophys. Acta* **112,** 403–421.
22. Armond, P. A. & Arntzen, C. J. (1977) *Plant Physiol.* **59,** 398–404.
23. Arntzen, C. J., Dilley, R. A., Peters, G. A. & Shaw, E. R. (1972) *Biochim. Biophys. Acta* **256,** 85–107.
24. Brown, J. S. (1973) in *Current Topics in Photophysiology,* ed. Giese, A. (Academic, New York), Vol. 8, pp. 97–111.
25. Akoyunoglou, G. (1978) in *Chloroplast Development,* eds. Akoyunoglou, G. & Argyroudi-Akoyunoglou, J. H. (Elsevier, Amsterdam), pp. 355–366.
26. Melis, A. & Akoyunoglou, G. (1977) *Plant Physiol.* **59,** 1156–1160.
27. Hall, D. O., Edge, H. & Kalina, M. (1971) *J. Cell Sci.* **9,** 289–303.
28. Nir, I. & Pease, D. C. (1973) *J. Ultrastruct. Res.* **42,** 534–550.
29. Park, R. B. & Sane, P. V. (1971) *Annu. Rev. Plant Physiol.* **22,** 395–430.