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Spectroscopic Methods in Photosynthesis: Photosystem Stoichiometry and Chlorophyll Antenna Size

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Spectroscopic methods in photosynthesis: photosystem stoichiometry and chlorophyll antenna size

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Light-induced absorbance change and fluorescence measurements were employed in the quantitation of photosystem stoichiometry and in the measurement of the chlorophyll (Chl) antenna size in thylakoid membranes. Results with thylakoid membranes from diverse photosynthetic tissues indicated a PSII/PSI reaction-centre stoichiometry that deviates from unity. Cyanobacteria and red algae have a PSII/PSI ratio in the range of 0.3 to 0.7. Chloroplasts from spinach and other vascular-plant species grown under direct sunlight have PSII/PSI = 1.8 ± 0.3. Chlorophyll *b*-deficient and Chl *b*-lacking mutants have PSII/PSI > 2. The observation that PSII/PSI ratios are not unity and show a large variation among different photosynthetic membranes appears to be contrary to the conventional assumption derived from the Z-scheme. However, the photosystem stoichiometry is not the only factor that needs to be taken into account to explain the coordination of the two photosystems in the process of linear electron transport. The light-harvesting capacity of each photosystem must also be considered. In cyanobacterial thylakoids (from *Synechococcus* 6301, PSII/PSI = 0.5 ± 0.2), the phycobilisome–PSII complexes collectively harvest as much light as the PSI complexes. In vascular plant chloroplasts, the light-harvesting capacity of a PSII complex (250 molecules, Chl *a*/Chl *b* = 1.7) is lower than that of a PSI complex (230 Chl, Chl *a*/Chl *b* = 8.0) because Chl *b* has a lower extinction coefficient than Chl *a*. A differential attenuation of light intensity through the grana further reduces the light absorbed by PSII. Hence, a PSII/PSI ratio greater than one in vascular-plant chloroplasts compensates for the lower absorption of light by individual PSII complexes and ensures that, on average, PSII will harvest about as much light as PSI. In conclusion, distinct light-harvesting strategies among diverse plant species complement widely different photosystem stoichiometries to ensure a balanced absorption of light and a balanced electron flow between the two photoreactions, thereby satisfying the requirement set forth upon the formulation of the Z-scheme by Hill & Bendall (*Nature*, *Lond.* **186**, 136–137 (1960)) and by Duysens, Amesz & Kamp (*Nature*, *Lond.* **190**, 510–511 (1961)).

Introduction

In oxygenic photosynthesis, electron transport occurs in the thylakoid membrane and requires coordinated interaction between a large number of electron-carrier compounds and enzymatic proteins that facilitate the transfer of electrons (reducing power) from dissociated H_2O molecules to $NADP^+$. The electron-transport components are highly organized in the thylakoid membrane and facilitate the transfer of electrons laterally in the plane of the membrane from the grana regions (appressed areas) to the stroma-exposed (unappressed) regions. Functionally, electron transport occurs from intermediate to intermediate in a sequential manner, formulated as the Z-scheme of electron transport by Hill & Bendall (1960).

The overall process of electron transport from H_2O to $NADP^+$ is strongly endergonic and is realized through the utilization of light energy at two discrete electron-transport steps occurring at photosystem II (PSII) and photosystem I (PSI) (Duysens *et al.* 1961). Implicit in the original hypothesis was the assumption that optimal electron flow in the electron-transport chain would occur if the two photosystems existed in equal quantities. This assumption of a 1:1 stoichiometric ratio between PSII and PSI was not rigorously tested for about 20 years. The advent of sensitive spectroscopic methods for the quantification of integral components within each photosystem provided detailed information on the question of photosystem stoichiometry in oxygenic photosynthesis. The assumption of a PSII/PSI = 1.0 ratio was not confirmed. The development of a kinetic method for the measurement of the light-harvesting chlorophyll antenna size of each photosystem helped provide an explanation for the variable photosystem stoichiometry.

This paper offers an overview of the application of spectrophotometric and kinetic methods for the investigation of photosystem stoichiometry and photosystem antenna size.

Materials and methods

Chlorophyll *a* (Chl *a*) fluorescence and absorbance difference measurements were made with a laboratory-constructed split-beam spectrophotometer with a resolution of less than 10^{-4} ΔA in the ultraviolet region of the spectrum (Melis & Hart 1980). Actinic excitation of a uniform field was provided in the green region of the spectrum, transmitted by a combination of CS 4–96 and CS 3–69 Corning glass filters. Green light was used to provide as equal as possible excitation of both Chl *a* and Chl *b* molecules (Melis & Anderson 1983; Ghirardi & Melis 1984).

Results and discussion

Spectrophotometric methods

Isolated thylakoid membranes can be electrochemically poised in the dark so that a subsequent illumination will induce primary charge separation in the reaction centres of PSI and PSII. The ensuing oxidation–reduction reactions can be monitored spectrophotometrically in a wavelength region specific to the molecule undergoing the redox transition. Figure 1 provides examples of the light-induced absorbance difference spectra generated upon the photoreduction of the specialized quinone acceptor Q_A of PSII (figure 1*a*), upon the photoreduction of the primary electron acceptor pheophytin of PSII (figure 1*b*) and upon the photooxidation of the reaction centre P_{700} of PSI (figure 1*c*). From the known differential extinction coefficients (Van Gorkom 1974; Demeter *et al.* 1987; Hiyama & Ke 1972) and from the amplitude of the absorbance change at the peak wavelength (320 nm for Q_A^-, 685 nm for $Pheo^-$ and 700 nm for P_{700}^+), it is possible to calculate the concentration of each chemical species in the thylakoid membrane, assuming that illumination of thylakoid membranes will result in the generation of one Q_A^- and one $Pheo^-$ per PSII centre, and in the generation of one P_{700}^+ per PSI centre. Hence it is possible to provide a direct estimate of the photosystem concentration in thylakoid membranes. Typical light-induced absorbance change traces with spinach thylakoids are shown in figure 2. The amplitude of the absorbance change at 700 nm (ΔA_{700}) indicated the presence of one P_{700} per 615 Chl ($a+b$) molecules. The amplitudes of the absorbance changes at 320 nm (ΔA_{320}) and 685 nm (ΔA_{685}) both indicated the presence of one

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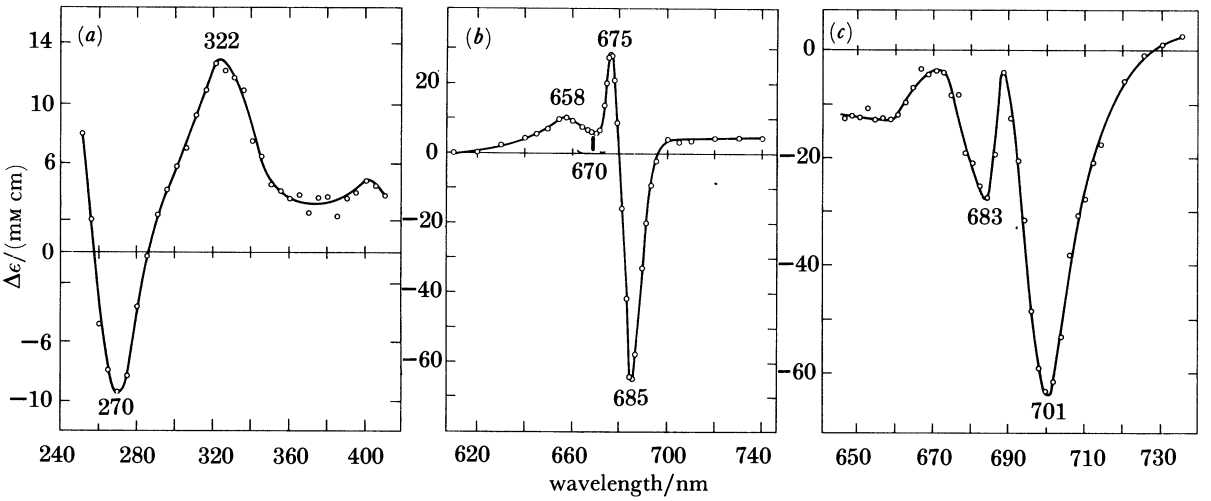

FIGURE 1. Light-induced absorbance difference spectra (*a*) of the reduced minus oxidized forms of the primary quinone acceptor of PSII ($Q_A^- - Q_A$), (*b*) of the reduced minus oxidized form of the primary electron acceptor pheophytin of PSII, and (*c*) of the oxidized minus reduced forms of the reaction centre P_{700} of PSI. The differential extinction coefficient values ($\Delta\epsilon$) were based on the work by Bensasson & Land (1973) and by Van Gorkom (1974) for $Q_A^- - Q_A$, by Ke *et al.* (1982) and by Demeter *et al.* (1987) for $Pheo^- - Pheo$, and by Hiyama & Ke (1972) for $P_{700}^+ - P_{700}$.

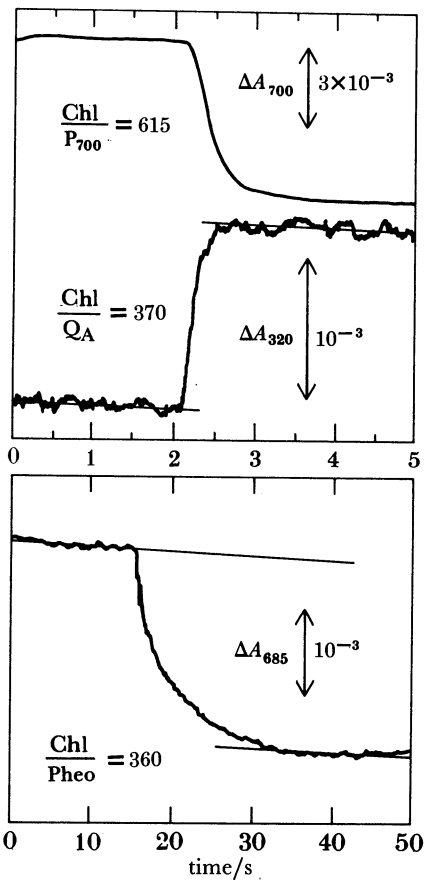

FIGURE 2. Light-induced absorbance difference change at 700 nm (ΔA_{700}), at 320 nm (ΔA_{320}) and at 685 nm (ΔA_{685}) attributed to the photo-oxidation of P_{700} and to the photoreduction of Q_A and pheophytin, respectively. The molar ratio of chlorophyll to component is given. Actinic excitation for the P_{700} and Q_A measurements came on at about 2.2 s. For the pheophytin measurement, actinic excitation came on at about 15 s.

PSII reaction centre (Q_A or Pheo) per 360–370 Chl ($a+b$) molecules. These measurements suggested a photosystem stoichiometry PSII:PSI = 1.7:1.0, i.e. there are more PSII than PSI reaction centres in the thylakoid membrane of vascular-plant chloroplasts (Melis & Brown 1980; Melis & Harvey 1981; Melis & Anderson 1983).

Further measurements with thylakoid membranes from diverse photosynthetic species indicated variability in the ratio of PSII and PSI reaction centres. Cyanobacteria and red algae commonly displayed photosystem stoichiometry PSII/PSI ratios between 0.3 and 0.7 (table 1) (Kawamura *et al.* 1979; Myers *et al.* 1980; Melis & Brown 1980; Manodori *et al.* 1984). Vascular-plant chloroplasts from spinach and from other species grown under direct sunlight have PSII/PSI ratios of 1.8±0.3 (Melis & Harvey 1981; Melis *et al.* 1985). Chlorophyll *b*-deficient mutants (Melis & Thielen 1980; Thielen & Van Gorkom 1981; Abadia *et al.* 1985; Ghirardi *et al.* 1986) and developing chloroplasts (Melis 1984; Tzinas *et al.* 1987; Glick & Melis 1988) display PSII/PSI ratios greater than two (table 1).

TABLE 1. CHLOROPHYLL RATIOS AND PHOTOSYSTEM STOICHIOMETRY IN CYANOBACTERIA, MATURE VASCULAR-PLANT CHLOROPLASTS, Chl *b*-DEFICIENT MUTANTS AND DEVELOPING CHLOROPLASTS

(Component quantification (molar ratio) is based on the Chl ($a+b$) content.)

	chlorophyll *a* / chlorophyll *b*	chlorophyll / PSI	chlorophyll / PSII	PSII / PSI
Synechococcus 6301	—	160	370	0.43
vascular plant chloroplast, wild type[a]	2.8	600	350	1.7
tobacco *Su/su*	4.7	330	120	2.7
barley *chlorina f2*	∞	300	100	3.0
intermittent-light developing plastids	∞	250	60	4.1

[a] Representative of wild type spinach, pea, barley, tobacco.

The experimental evidence that photosystem stoichiometry ratios (PSII/PSI) differed from 'strict unity' (and showed large variations among different photosynthetic membranes) was contrary to the conventional dogma interpreted from the Z-scheme and was not readily accepted (De Vitry *et al.* 1983; Whitmarsh & Ort 1984*a*, *b*). However, optimal and efficient electron flow between the two photosystems requires statistically equal rates of light absorption and light utilization by the two photoreactions, rather than equal photosystem stoichiometries.

The evaluation of excitation-energy distribution between the two photosystems requires an analysis of the different light-harvesting strategies among diverse photosynthetic organisms. This analysis should take into consideration the photosystem stoichiometry, the size and composition of the light-harvesting pigments of each photosystem, light absorption in the grana against that in the stroma-exposed thylakoids of vascular plant chloroplasts, and the absorption of light by phycobilisomes (PBSs) in cyanobacteria and red algae. A limited assessment of the effect of these parameters for spinach chloroplasts and for cyanobacteria is presented here.

Kinetic methods

The absolute size of the Chl antenna of PSI and PSII can be determined from the known ratio of total Chl to a reaction centre (Chl/PSI andChl/PSII, figure 2 and table 1) upon

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apportioning this total Chl into distinct PSI and PSII components. The approach employed by Melis and co-workers was to assign Chl to each reaction centre in direct proportion to the rate of light utilization by each photosystem. The premise of this approach is that, under light-limiting conditions, the rate of photochemistry is directly proportional to the light-harvesting antenna size. It was implemented by comparing the kinetics of the fluorescence induction curve of chloroplasts in the presence of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) with the kinetics of ΔA_{700} of chloroplasts poisoned with KCN under broad-band green light of limiting intensity (Melis & Anderson 1983; Ghirardi & Melis 1984). In the presence of DCMU, the reoxidation of Q_A^- by Q_B is inhibited and the fluorescence induction kinetics provide a measure of the rate of trap closure and hence a measure of the rate of light absorption by the antenna of PSII (Duysens & Sweers 1963). In KCN-poisoned chloroplasts, the function of plastocyanin is inhibited (Ouitrakul & Izawa 1973); thus electron donation to P_{700}^+ is prevented. Under these conditions, the kinetics of ΔA_{700} provide a measure of the rate of light absorption by the antenna pigments of PSI. Figure 3 compares the kinetics of the absorbance decrease at 700 nm (ΔA_{700}) in KCN-poisoned chloroplasts (reflecting the photooxidation of P_{700}) with the fluorescence induction kinetics in DCMU-poisoned chloroplasts (reflecting the photoreduction of Q_A). The semi-logarithmic plots in figure 4 provide a quantitative comparison of the kinetic data. The photooxidation of P_{700} is a monophasic exponential function of time, as evidenced by the single straight line in the semi-logarithmic plot (figure 4*a*), revealing the existence of a uniform population of PSI antenna size. The slope of this line defined the rate constant of light absorption by PSI ($K_I = 10.0$ photons s^{-1}).

The photoreduction of Q_A occurs with biphasic kinetics revealing, in vascular-plant chloroplasts, the existence of two populations of PSII centres, $PSII_\alpha$ and $PSII_\beta$ (Melis &

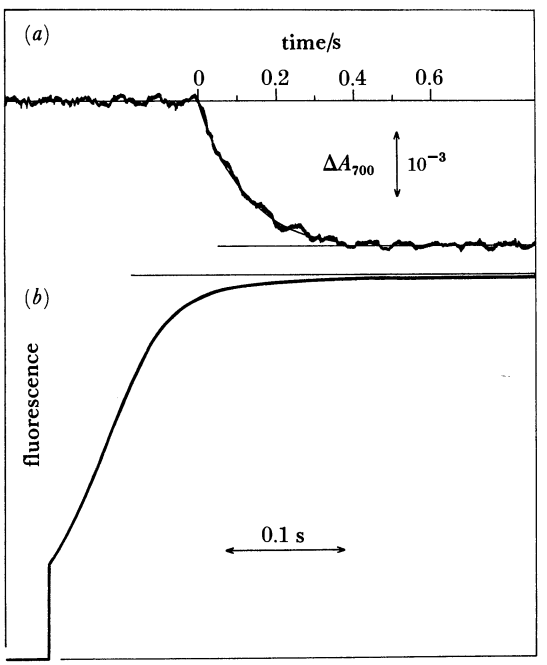

FIGURE 3. (*a*) The timecourse of the absorbance change at 700 nm (ΔA_{700}) monitored with potassium cyanide-poisoned chloroplasts in the presence of 200 μM methyl viologen. (*b*) The fluorescence induction curve of isolated chloroplasts in the presence of 20 μM DCMU and 1 mM hydroxylamine.

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Homann 1976) that differ on the basis of antenna size. Figure 4*b* (open circles) illustrates the biphasic nature of photochemistry at PSII. The slow linear phase, attributed to the photoactivity of $PSII_\beta$, is a monophasic exponential function of time, the slope of which defined the rate constant, K_β, equal to 5.0 photons s^{-1}. The fast phase of PSII photoactivity is non-first-order and its kinetics are determined by subtracting the slow exponential phase from the overall kinetic phenomenon (figure 4*b*, solid circles). The rate constant K_α of $PSII_\alpha$ was estimated from the slope of the semilogarithmic plot at zero time to be 11.0 photons s^{-1}.

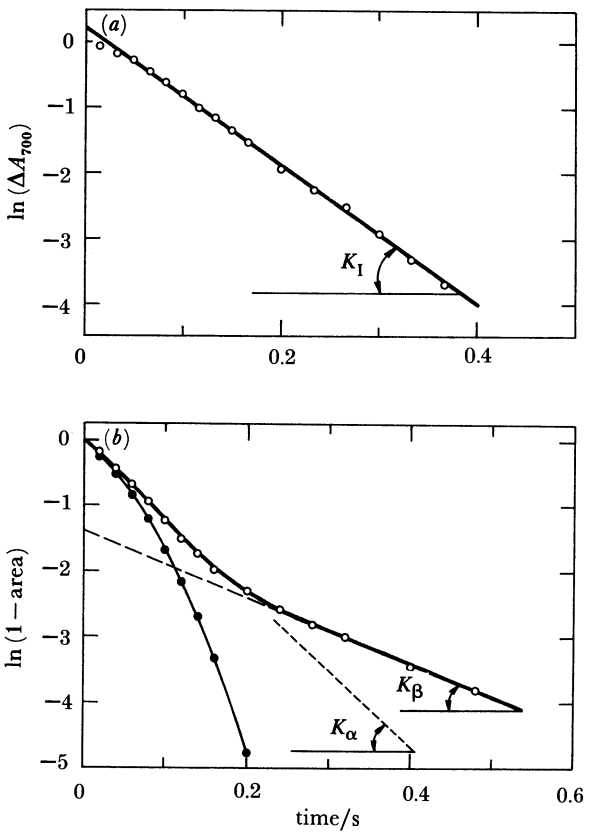

FIGURE 4. (*a*) A semi-logarithmic plot of the kinetics of ΔA_{700} showing the monophasic exponential function of time for P_{700} photooxidation. The slope of the straight line defines the rate of light utilization by PSI, K_I. (*b*) A semi-logarithmic plot of the kinetics of the area over the fluorescence induction curve (open circles). The slope of the slower linear phase defined the rate constant K_β of $PSII_\beta$ photoreduction. The semilogarithmic plot of the kinetics of $PSII_\alpha$ photoreduction (solid circles) deviated from linearity. The rate constant K_α was determined from the slope of the nonlinear curve (solid circles) at zero time.

The intercept of the slower β-phase with the ordinate at zero time (dashed line in figure 4*b*) provided a measure of the relative proportion of $PSII_\beta$ in the thylakoid lamellae of spinach chloroplasts. It was estimated that $PSII_\beta$ accounted for about 25% of the total PSII reaction centres and $PSII_\alpha$ accounted for the remaining 75%. As the overall PSII:PSI reaction-centre ratio in spinach is 1.7:1.0 (table 1), it follows that $PSII_\alpha:PSII_\beta:PSI = 1.3:0.4:1.0$.

From these ratios and from the experimentally determined values of the rate constants K_α, K_β and K_I, the absolute numbers, N_α, N_β and N_I, of chlorophyll molecules transferring excitation

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to the reaction centres of $PSII_\alpha$, $PSII_\beta$ and PSI, respectively, were determined. This was accomplished from the solution of the following system of equations:

$$\text{Chl/PSI} = N_\alpha \text{PSII}_\alpha/\text{PSI} + N_\beta \text{PSII}_\beta/\text{PSI} + N_\text{I} \quad (1)$$

$$K_\alpha = cN_\alpha \quad (2)$$

$$K_\beta = cN_\beta \quad (3)$$

$$K_\text{I} = cN_\text{I}, \quad (4)$$

where Chl/PSI is the ratio of the total Chl $(a+b)$ per PSI reaction centre and c is a proportionality constant that depends on the quantum yield of photochemistry at each photosystem. Although the quantum yield of photochemistry at the three photosystems is not unity, it is generally accepted to be greater than 0.8 (Avron & Ben-Hayyim 1969; Sun & Sauer 1971; Thielen & Van Gorkom 1981; Ley & Mauzerall 1982). For the solution of equations (1)–(4) we assumed that the quantum yield for photochemistry at $PSII_\alpha$, PSII and PSI are similar, thereby using the same value of c (Thielen & Van Gorkom 1981; Melis & Anderson 1983). The Chl antenna sizes of $PSII_\alpha$ ($N_\alpha = 250$), of $PSII_\beta$ ($N_\beta = 115$) and of PSI ($N_\text{I} = 230$) are summarized in table 2.

TABLE 2. PHOTOSYSTEM STOICHIOMETRY AND CHLOROPHYLL DISTRIBUTION IN SUN-ADAPTED VASCULAR-PLANT CHLOROPLASTS AND IN CYANOBACTERIA

	stoichiometry	Chl antenna size	Chl a / Chl b	Chl b / Chl $(a+b)$ (%)
vascular plants				
$PSII_\alpha$	1.3	250	155:95	38
$PSII_\beta$	0.4	115	85:30	26
PSI	1.0	230	205:25	11
cyanobacteria				
PSII	0.5	35	—	—
PSI	1.0	140	—	—

The same technique, when applied to the thylakoid membranes of cyanobacteria (*Synechococcus* 6301) revealed monophasic PSII kinetics, suggesting the absence of heterogeneity in the Chl antenna of this organism. Measurements yielded a PSII antenna size of 35 Chl *a* molecules ($N_\text{II} = 35$ Chl *a*) and a PSI antenna size of 140 Chl *a* molecules ($N_\text{I} = 140$ Chl *a*) (table 2).

Excitation-energy distribution

Cyanobacteria

The photochemical apparatus organization and photosystem stoichiometry in the thylakoid membrane of *Synechococcus* 6301 is schematically shown in figure 5 (Manodori *et al.* 1984). Each PSI complex contains a light-harvesting antenna of 140 Chl *a* molecules whereas PSII complexes contain only about 35 Chl *a* molecules. The lower stoichiometric ratio (PSII/PSI $= 0.5 \pm 0.2$, table 2) and the lower Chl *a* antenna size ($N_\text{II} = 35$, $N_\text{I} = 140$) of PSII in cyanobacteria is corrected thanks to light absorption by the PSII-associated phycobilisomes (PBSs). Each PBS contains approximately 400 bilins (Manodori *et al.* 1984) organized in the

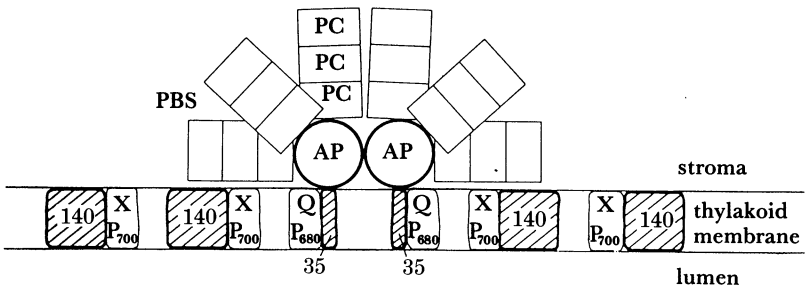

FIGURE 5. Photochemical apparatus organization and photosystem stoichiometry in *Synechococcus* 6301. The thylakoid membrane integral components include the reaction-centre complexes of PSI (P_{700}–X) and PSII (P_{680}–Q), each associated with a distinct Chl-protein light-harvesting complex. The light-harvesting antenna of PSI contains approximately 140 Chl *a* molecules, that of PSII about 35. The phycobilisome (PBS) is loosely bound to the Chl–protein of two PSII complexes via the core cylinders. Each PBS contains about 400 bilin molecules organized in the form of phycocyanin (PC) in the peripheral rods and allophycocyanin (AP) complexes in the core cylinders.

form of phycocyanin (PC) and allophycocyanin (AP) complexes in the peripheral rods and core cylinders, respectively (see figure 5) (Glazer 1982). Two PSII complexes, each functionally connected to one of the PBS core cylinders (Manodori & Melis 1985), compete for excitation energy from the same PBS. On the basis of this structural and functional organization of the photochemical apparatus in cyanobacteria, the rate of light absorption by the two photosystems can be estimated. Figure 6 shows the absorbance spectrum of intact *Synechococcus* 6301 cells (solid line) and, upon deconvolution, the absorbance spectra of PBS–PSII (dashed line) and of PSI (dotted lines) in the cell. The integrated absorbance of light by the PBS–PSII complexes (area defined by the dashed trace) is approximately equal to the integrated absorbance of light by the PSI complexes in the cell (area defined by the dotted trace), supporting the notion that overall absorption of light by the PBS–PSII complexes in *Synechococcus* 6301 cells is equal to that of the PSI complexes.

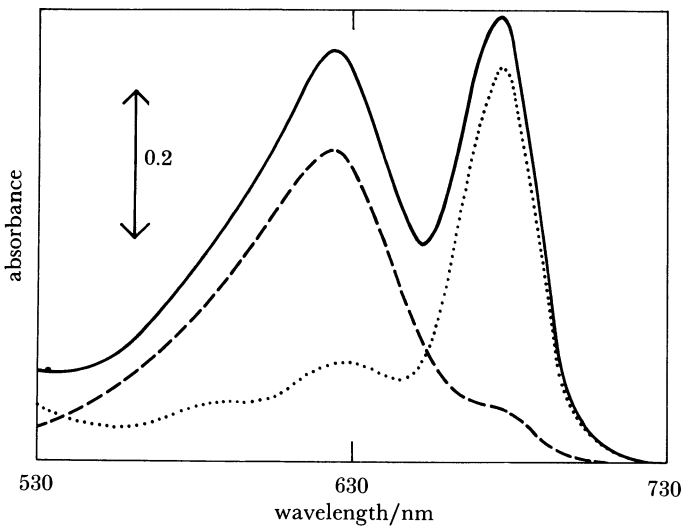

FIGURE 6. Absorbance spectra of *Synechococcus* 6301 cells (———) and the partial spectra of PBS–PSII (-----) and PSI (.....) in the cell. The partial spectra were obtained upon deconvolution of the *in vivo* absorbance spectrum of *Synechococcus* 6301.

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The above results provide a rationale for understanding the low PSII/PSI (less than 1) stoichiometry in cyanobacteria and suggest that the photosystem stoichiometry need not be equal to 1.0 to ensure an overall balanced absorption of light by PSII and PSI complexes in the thylakoid membrane.

Wild-type vascular-plant chloroplasts

A summary of the photochemical apparatus organization and photosystem stoichiometry in vascular-plant chloroplasts (spinach, pea, tobacco, barley) is presented in table 2. The analysis revealed a significantly greater PSII than PSI concentration in the thylakoid membrane and a functional Chl antenna size which is slightly larger for PSII than for PSI. In summary, about 60 % of all Chl is associated with PSII and only about 40 % is associated with PSI. The uneven association of Chl with the two photosystems may convey the impression that light absorption by PSII will exceed that by PSI, resulting in dissimilar rates of light utilization by the two photoreactions.

However, a thorough understanding of the coordination of the two photosystems in the process of light absorption in chloroplasts requires further analysis of the efficiency of light absorption by PSII and PSI under *in vivo* conditions. The latter includes understanding (*a*) the effect of Chl *b* on the rate of light absorption by individual PSII and PSI complexes (molecular level), and (*b*) the loss of light absorption by PSII due to the segregation of $PSII_\alpha$ in the grana stacks where the density of light-absorbing pigment is relatively high (membrane level).

(*a*) *The rate of light absorption by individual PSII and PSI complexes.* Although Chl *a* and Chl *b* absorb light both in the red and in the blue region of the spectrum, action spectra of photosynthesis suggest that red light is markedly more efficient than blue light in driving photochemistry (Emerson & Lewis 1943; Oquist 1969; Bazzaz & Govindjee 1973). Hence it is of interest to provide an evaluation of the properties of light absorption by Chl *a* and Chl *b* in the wavelength region between 550 and 750 nm.

On the basis of known extinction-coefficient spectra (MacKinney 1940), it was determined that the integrated absorbance of light by Chl *a* is approximately 1.5 times that of Chl *b* in 80 % acetone. The integrated absorbance of light by Chl *a* is substantially greater than that of Chl *b* under *in vivo* conditions as well. This is evidenced in the results of figure 7, which shows the absorbance spectrum of purified light-harvesting complex II (LHCII) from spinach. Two main absorbance maxima are discerned, one at 675 nm (predominantly due to Chl *a*), and another at 652 nm (predominantly due to Chl *b*) (Ryrie *et al.* 1980). The Chl *a*/Chl *b* (mol:mol) ratio of the preparation was approximately 1.1:1.0. In spite of the approximately equimolar amounts of Chl *a* and Chl *b* in the LHCII, the Chl *b* absorbance maximum is substantially lower than that of Chl *a*. This argues in favour of a significantly lower extinction coefficient *in vivo* for Chl *b* than for Chl *a*. As about 80 % of the total Chl *b* is associated with the antenna of $PSII_\alpha$ in the grana partition regions (Andersson & Anderson 1980; Melis & Anderson 1983), it was estimated that a $PSII_\alpha$ complex, in spite of the numerical superiority of Chl molecules in its antenna, would absorb about 5 % less light than a PSI complex (Melis *et al.* 1987).

(*b*) *Loss of light absorption due to granal membrane appression.* The evidence presented above shows that, at the molecular level, PSII is handicapped in terms of light absorption because of the substantial amounts of Chl *b* contained in its light-harvesting antenna. It is known that $PSII_\alpha$ and the LHCII are segregated in the partition regions of grana (Andersson & Anderson 1980; Andersson & Haehnel 1982; Anderson & Melis 1983). Thus the grana stacks constitute

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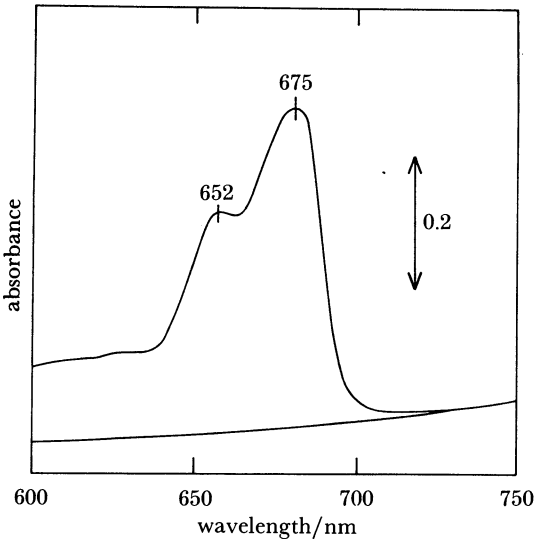

FIGURE 7. Absorbance spectra of resolved Chl *ab* light-harvesting complex of PSII (LHCII, Chl *a*/Chl *b* = 1.1) comparing the relative Chl *b* (652 nm) and Chl *a* (675 nm) peak heights.

areas of high pigment density where there is substantial attenuation of light transmission ('flattening' of absorbance). Quantification of this phenomenon has been provided by Jennings & Zuccheli (1985), who found that stacked thylakoids absorb about 20% less light than unstacked thylakoids. This loss of light absorption affects specifically $PSII_\alpha$ because PSI complexes are excluded from the membrane of the grana partition regions. It may be concluded that, at the thylakoid membrane level, PSII is handicapped in terms of light absorption because of the substantial attenuation of light transmission through the grana stacks where PSII is segregated.

In summary, a stoichiometric ratio $PSII_\alpha$:PSI = 1.3:1.0 (table 2) helps to compensate for the lower absorption of light by individual PSII complexes, and will result in a statistically balanced distribution of excitation between PSII and PSI in the thylakoid membrane. The contribution of $PSII_\beta$ to overall light absorption is small because of its low concentration and small antenna size. It was estimated that $PSII_\beta$ accounts for only about 7–8% of the total absorption of light by the chloroplast (Melis & Anderson 1983).

Chlorophyll b-deficient mutants and intermittent light (developing) plastids

Photosynthetic mutants deficient in Chl *b* and mutants lacking Chl *b* have enhanced PSII/PSI stoichiometry (table 1). A substantially enhanced PSII/PSI stoichiometry is also characteristic of intermittent-light (developing) plastids. Chloroplasts in these various organisms possess fully functional photochemical reaction centres and other electron-transport intermediates. However, they show Chl *a*/Chl *b* ratios that are significantly higher than the corresponding wild type and often show altered thylakoid-membrane ultrastructure. A deficiency or lack of Chl *b* from the thylakoid membrane results in smaller photosynthetic unit size in both photosystems (Thielen & Van Gorkom 1981; Ghirardi *et al.* 1986; Glick & Melis 1988). However, because about 80% of Chl *b* is associated with PSII, it follows that in Chl *b*-deficient and in Chl *b*-less chloroplasts, the light-harvesting capacity of PSII is lowered substantially more than that of PSI. Table 3 shows the results of photosystem stoichiometry and

Table 3. Photosystem stoichiometry and chlorophyll distribution in the Chl *b*-less *chlorina f*2 chloroplasts of barley and in barley plastids developing under intermittent illumination (ImL plastids)

	PSII/PSI stoichiometry	chlorophyll antenna size PSII	PSI
Chl *b*-less *chlorina f*2 (barley)	3.0	50	150
ImL plastids	4.1	37	95

Chl antenna size measurements with chloroplasts from the Chl *b*-less *chlorina f*2 mutant of barley and with plastids developing under intermittent illumination.

The absence of Chl *b* from the *chlorina f*2 chloroplasts resulted in a drastic reduction of the PSII functional antenna size from about 250 Chl (*a*+*b*) in the wild type to about 50 Chl *a* in the mutant, i.e. a reduction by about 80% in the number of Chl molecules specifically associated with PSII. In addition, the kinetic differentiation of PSII into $PSII_\alpha$ and $PSII_\beta$ was no longer evident in the mutant (Percival *et al.* 1984; Ghirardi *et al.* 1986). By comparison, the functional antenna size of PSI in the *chlorina f*2 was smaller by only about 25%. Qualitatively similar results were obtained with intermittent light plastids (table 3). The measurement of the Chl antenna size of PSII and PSI in Chl *b*-deficient and Chl *b*-less chloroplasts provide a rationale for understanding the elevated PSII/PSI stoichiometry in these samples. It may be argued that the significantly enhanced PSII/PSI reaction-centre ratio in these samples is designed to compensate for the substantially smaller PSII antenna size so that an overall parity in light absorption and electron transport between PSII and PSI is maintained (Melis *et al.* 1985).

Conclusion

Implicit in the formulation of the Z-scheme is the assumption of a balanced electron flow between PSII and PSI centres in the thylakoid membrane of oxygenic photosynthesis (Hill & Bendall 1960; Duysens *et al.* 1961). Measurements of photosystem stoichiometry and photosystem antenna size, summarized above, suggest that the above principle (the principle of Hill & Bendall and of Duysens *et al.*) is satisfied in spite of the highly divergent photosystem stoichiometry, pigment composition and properties of light absorption by the photosystems among several genera of photosynthesis organisms. Balanced electron flow between the two photosystems requires approximately equal utilization of light by the two photoreactions, rather than equal photosystem stoichiometry.

References

Abadia, J., Glick, R. E., Taylor, S. E., Terry, N. & Melis, A. 1985 Photochemical apparatus organization in the chloroplasts of two *Beta vulgaris* genotypes. *Pl. Physiol.* **79**, 872–878.

Anderson, J. M. & Melis, A. 1983 Localization of different photosystems in separate regions of chloroplast membranes. *Proc. natn. Acad. Sci. U.S.A.* **80**, 745–749.

Andersson, B. & Anderson, J. M. 1980 Lateral heterogeneity in the distribution of chlorophyll–protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim. biophys. Acta* **593**, 427–440.

Andersson, B. & Haehnel, W. 1982 Location of photosystem I and photosystem II reaction centres in different thylakoid regions of stacked chloroplasts. *FEBS Lett.* **146**, 13–17.

Avron, M. & Ben-Hayyim, G. 1969 Interaction between two photochemical systems in photoreactions of isolated chloroplasts. In *Progress in photosynthesis research* (ed. H. Metzner), vol. 3, pp. 1185–1196. Tubingen: H. Laupp.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bazzaz, M. B. & Govindjee 1973 Photochemical properties of mesophyll and bundle sheath chloroplasts of maize. *Pl. Physiol.* **52**, 257–262.

Bensasson, R. & Land, E. J. 1973 Optical and kinetic properties of semireduced plastoquinone and ubiquinone: electron acceptors in photosynthesis. *Biochim. biophys. Acta* **325**, 175–181.

De Vitry, C., Wollman, F. A. & Delepelaire, P. 1983 Stoichiométrie des polypeptides du thylakoide chez *Chlamydomonas reinhardtii*. *C. R. Séanc. Acad. Sci., Paris* **297**, 277–280.

Demeter, S., Neale, P. J. & Melis, A. 1987 Photoinhibition: impairment of the primary charge separation between P680 and pheophytin in photosystem II of chloroplasts. *FEBS Lett.* **214**, 370–374.

Duysens, L. N. M. & Sweers, H. E. 1963 Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In *Microalgae and photosynthetic bacteria*, pp. 353–372. University of Tokyo Press.

Duysens, L. N. M., Amesz, J. & Kamp, B. M. 1961 Two photochemical systems in photosynthesis. *Nature, Lond.* **190**, 510–511.

Emerson, R. & Lewis, C. M. 1943 The dependence of the quantum yield of *Chlorella* photosynthesis on wave length of light. *Am. J. Bot.* **30**, 165–178.

Ghirardi, M. L. & Melis, A. 1984 Photosystem electron-transport capacity and light-harvesting antenna size in maize chloroplasts. *Pl. Physiol.* **74**, 993–998.

Ghirardi, M. L., McCauley, S. W. & Melis, A. 1986 Photochemical apparatus organization in the thylakoid membrane of *Hordeum vulgare* wild type and chlorophyll *b*-less *chlorina f*2 mutant. *Biochim. biophys. Acta* **851**, 331–339.

Glazer, A. N. 1982 Phycobilisomes, structure and dynamics. *A. Rev. Microbiol.* **36**, 173–198.

Glick, R. E. & Melis, A. 1988 Minimum photosynthetic unit size in system I and system II of barley chloroplasts. *Biochim. biophys. Acta* **934**, 151–155.

Hill, R. & Bendall, F. 1960 Function of the two cytochrome components in chloroplasts. A working hypothesis. *Nature, Lond.* **186**, 136–137.

Hiyama, T. & Ke, B. 1972 Difference spectra and extinction coefficients of P700. *Biochim. biophys. Acta* **267**, 160–171.

Jennings, R. C. & Zucchelli, G. 1985 The influence of membrane stacking on light absorption by chloroplasts. *Photobiochem. Photobiophys.* **9**, 215–221.

Kawamura, M., Mimuro, M. & Fujita, Y. 1979 Quantitative relationship between two reaction centres in the photosynthetic system of blue-green algae. *Pl. Cell Physiol.* **20**, 697–705.

Ke, B., Inoue, H., Babcock, G. T., Fang, Z. X. & Dolan, E. 1982 Optical and EPR characterization of oxygen-evolving photosystem II subchloroplast fragments isolated from the thermophilic blue-green alga *Phormidium laminosum*. *Biochim. biophys. Acta* **682**, 297–306.

Ley, A. C. & Mauzerall, D. C. 1982 Absolute absorption cross sections for photosystem II and the minimum quantum requirement for photosynthesis in *Chlorella vulgaris*. *Biochim. biophys. Acta* **680**, 95–106.

MacKinney, G. 1940 Criteria for purity of chlorophyll preparations. *J. biol. Chem.* **132**, 91–109.

Manodori, A., Alhadeff, M., Glazer, A. & Melis, A. 1984 Photochemical apparatus organization in *Synechococcus* 6301 (*Anacystis nidulans*). Effect of phycobilisome mutation. *Arch. Microbiol.* **139**, 117–123.

Manodori, A. & Melis, A. 1985 Phycobilisome–photosystem II association in *Synechococcus* 6301. *FEBS Lett.* **181**, 79–82.

Melis, A. 1984 Light regulation of photosynthetic membrane structure, organization and function. *J. biol. Chem.* **24**, 271–285.

Melis, A. & Anderson, J. M. 1983 Structural and functional organization of the photosystems in spinach chloroplasts. Antenna size, relative electron-transport capacity and chlorophyll composition. *Biochim. biophys. Acta* **724**, 473–484.

Melis, A. & Brown, J. S. 1980 Stoichiometry of system I and system II reaction centres and of plastoquinone in different photosynthetic membranes. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4712–4716.

Melis, A. & Hart, R. W. 1980 A laboratory-constructed sensitive difference spectrophotometer for the ultraviolet, visible, and far-red region of the spectrum. *Carnegie Instn. Wash. Yb.* **79**, 170–172.

Melis, A. & Harvey, G. W. 1981 Regulation of photosystem stoichiometry, chlorophyll *a* and chlorophyll *b* content and relation to chloroplast ultrastructure. *Biochim. biophys. Acta* **637**, 138–145.

Melis, A. & Homann, P. H. 1976 Heterogeneity of the photochemical centres in system II of chloroplasts. *Photochem. Photobiol.* **23**, 343–350.

Melis, A., Manodori, A., Glick, R. E., Ghirardi, M. L., McCauley, S. W. & Neale, P. J. 1985 The mechanism of photosynthetic membrane adaptation to environmental stress conditions: a hypothesis on the role of electron-transport capacity and of ATP/NADPH pool in the regulation of thylakoid membrane organization and function. *Physiol. veg.* **23**, 757–765.

Melis, A., Spangfort, M. & Anderson, B. 1987 Light-absorption and electron-transport balance between photosystem II and photosystem I in spinach chloroplasts. *Photochem. Photobiol.* **45**, 129–136.

Melis, A. & Thielen, A. P. G. M. 1980 The relative absorption cross-sections of photosystem I and photosystem II in chloroplasts from three types of *Nicotiana tabacum*. *Biochim. biophys. Acta* **589**, 275–286.

Myers, J., Graham, J. R. & Wang, R. T. 1980 Light-harvesting in *Anacystis nidulans* studied in pigment mutants. *Pl. Physiol.* **66**, 1144–1149.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Oquist, G. 1969 Adaptations in pigment composition and photosynthesis by far-red radiation in *Chlorella pyrenoidosa*. *Physiologia Pl.* **22**, 516–528.

Ouitrakul, R. & Izawa, S. 1973 Electron-transport and photophosphorylation in chloroplasts as a function of the electron acceptor. II. Acceptor-specific inhibition by KCN. *Biochim. biophys. Acta* **305**, 105–118.

Percival, M. P., Webber, A. N. & Baker, N. R. 1984 Evidence for the role of the light-harvesting chlorophyll *a*/*b* protein complex in photosystem II heterogeneity. *Biochim. biophys. Acta* **767**, 582–589.

Ryrie, I. J., Anderson, J. M. & Goodchild, D. J. 1980 The role of the light-harvesting chlorophyll *a*/*b*-protein complex in chloroplast membrane stacking. *Eur. J. Biochem.* **107**, 345–354.

Sun, A. S. K. & Sauer, K. 1971 Pigment systems and electron transport in chloroplasts. *Biochim. biophys. Acta* **234**, 399–414.

Thielen, A. P. G. M. & Van Gorkom, H. J. 1981 Quantum efficiency and antenna size of photosystem II_α, II_β and I in tobacco chloroplasts. *Biochim. biophys. Acta* **635**, 111–120.

Tzinas, G., Argyroudi-Akoyunoglou, J. H. & Akoyunoglou, G. 1987 The effect of the dark interval in intermittent light on thylakoid development: photosynthetic unit formation and light-harvesting protein accumulation. *Photosynth. Res.* **14**, 241–258.

Van Gorkom, H. J. 1974 Identification of the reduced primary electron acceptor of photosystem II as a bound semiquinone anion. *Biochim. biophys. Acta* **347**, 439–442.

Whitmarsh, J. & Ort, D. R. 1984*a* Quantitative determination of the electron-transport complexes in the thylakoid membranes of spinach and several other plant species. In *Advances in photosynthesis research* (ed. C. Sybesma), vol. 3, pp. 231–234. Dortrecht: Martinus Nijhoff.

Whitmarsh, J. & Ort, D. R. 1984*b* Stoichiometries of electron transport complexes in spinach chloroplasts. *Archs. Biochem. Biophys.* **231**, 378–389.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES