Biochimica et Biophysica Acta, 935 (1988) 152–165
Elsevier

BBA 42834

Changes in the organization of stroma membranes induced by in vivo state 1–state 2 transition

Roberto Bassi [a], Giorgio M. Giacometti [a] and David J. Simpson [b]

[a] *Dipartimento di Biologia, Università di Padova, Padova (Italy) and* [b] *Department of Physiology, Carlsberg Laboratory, Valby (Denmark)*

(Received 14 March 1988)

Key words: Excitation energy transfer; Fluorescence emission spectroscopy; Light harvesting complex II; Thylakoid membrane; Photosystem I; Protein phorphorylation

Stroma lamellae were isolated from mesophyll thylakoids of maize plants exposed to dark (state 1) and high-intensity white light (state 2). Freeze-fracture electron microscopy established that the preparations consisted of small, spherical, right-side-out vesicles, and that the extent of contamination by grana appressed lamellae was less than 0.1%. The state 1–state 2 transition resulted in a decrease in the chlorophyll *a*/*b* ratio of the isolated stroma lamellae due to lateral migration of light-harvesting chlorophyll *a*/*b* protein of Photosystem II (LHC II) from appressed grana lamellae. Fluorescence spectroscopy showed that this 'mobile LHC II' became functionally attached to the Photosystem I reaction centre, with a resulting 15% increase in antenna size, as determined by measurement of the chlorophyll/P-700 ratio and kinetics of P-700 photo-oxidation under light-limiting conditions. The mobile LHC II could be resolved by gel electrophoresis into four polypeptides with an approximate 1:1:1:1 stoichiometry. This differed significantly from the polypeptide composition of grana LHC II, which had a different stoichiometry, and an additional polypeptide of lower apparent molecular mass which did not become phosphorylated. The freeze-fracture particle density data showed that the phosphorylated LHC II was not visible as discrete small particles, as found in appressed grana membranes, and may have become incorporated into the large PF particles of the stroma lamellae, which contain the Photosystem I reaction centre.

Introduction

Plant growth patterns under natural conditions, and their association into canopies, result in shading of shorter species and of the lower leaves of the same plant [1]. The illumination in such a situation is not constant, and an individual leaf can be exposed to full sunlight or deep shade within a short time. Shaded leaves receive light enriched in its far-red component, which is preferentially absorbed by Photosystem I (PS I), while in full sunlight relatively more of the excitation energy is absorbed by Photosystem II (PS II) [2]. Short-term changes in the extent of shading would therefore unbalance the energy distribution between the two photosystems, with a reduction in the electron transport rate and overall efficiency, unless a mechanism existed for the dynamic adaptation of the PS I and PS II cross-sections.

Abbreviations: EF, endoplasmic fracture face; LHC I, light-harvesting chlorophyll *a*/*b* protein of PS I; LHC II, light-harvesting chlorophyll *a*/*b* protein of PS II; PAGE, polyacrylamide gel electrophoresis; PF, protoplasmic fracture face; PS I, Photosystem I; PS II, Photosystem II; SDS, sodium dodecyl sulphate.

Correspondence: R. Bassi, Dipartimento di Biologia, Università di Padova, Via Loredan 10, 35100 Padova, Italy.

When leaves are put into darkness, or exposed to light which preferentially excites PS I, they undergo a transition to state 1 in which the light energy absorbed by the major light-harvesting chlorophyll-protein of PS II (LHC II) is preferentially transferred to PS II. This state is characterised by a high fluorescence yield. Under conditions in which light is preferentially absorbed by PS II, a transition to a low fluorescence state 2 is induced over a time-scale of several minutes, leading to a redistribution of excitation energy, allowing more light to be absorbed by PS I [3,4].

Although changes in the cation concentration in vitro have been shown to induce changes resembling state transitions [5,6], it is the phosphorylation of LHC II which ultimately regulates energy distribution between the two photosystems as a result of state transitions [7–10]. This phosphorylation is thought to be mediated by a thylakoid bound, Mg^{2+}-dependent kinase [11,12], whose activity is regulated by the redox state of the plastoquinone pool [8,13,14], which in turn reflects the excitation energy distribution between PS II and PS I. The addition of negatively charges phosphate groups to the LHC II complex causes it to dissociate from PS II, and favours its migration out of the appressed regions of the grana and into the stroma lamellae, where PS I centres are located [15].

The model above has been derived from experiments in which the phosphorylation of LHC II was induced in vitro using isolated chloroplasts or thylakoid membranes, either by suitable illumination, or by chemical alteration of the redox state of the plastoquinone pool. The purpose of the present work was to examine this model in an in vivo system, and to characterise the chlorophyll-protein complexes involved in the regulation of energy distribution. We induced state 1 or state 2 in whole leaves of maize by adaption of plant to the dark, or strong white light, respectively. Stroma lamellae were isolated in the presence of the phosphatase inhibitor, NaF, and were examined by spectroscopy, electrophoresis and freeze-fracture electron microscopy. We show that state 2 is associated with increased amounts of LHC II in the stroma, and that this LHC II has a polypeptide composition different from that of LHC II isolated from whole thylakoids or grana. We confirm the hypothesis of a mobile LHC II subpopulation [10] and demonstrate that this LHC II is able to transfer energy to PS I after lateral migration in vivo.

Materials and Methods

Plant material

Maize seeds (*Zea mays* L. cv. DeKalb DF 28) were soaked in water for 24 h and grown in a vermiculite/soil mixture under glasshouse conditions in summer. High light conditions were maintained by supplementing daylight with mercury vapour lamps.

Light adaptation and thylakoid isolation

One batch of 3-week-old maize plants was kept in the dark, while a second batch was exposed to bright light from a halogen metal vapour lamp (Osram HQI-E) producing a daylight spectrum with an intensity of 500 $\mu E/m^2$ per s. After 1 h adaptation, leaves were rapidly harvested and immediately ground up in a homogeniser with replaceable razor-blades [16], using 10 vol. of ice-cold buffer (350 mM sorbital/50 mM Hepes (pH 7.5)/10 mM $MgCl_2$/1 mM ascorbate/10 mM NaF). After 4×3 s full-speed bursts, mesophyll chloroplasts were obtained by filtering the resulting slurry through two layers of 30 μm nylon mesh and centrifuging for 10 min at $1500 \times g$ [17]. The pellet was resuspended in 50 mM Hepes (pH 7.5)/10 mM $MgCl_2$/1 mM ascorbate/10 mM NaF, and left to stand for 10 min at 0 °C. After 10 min centrifugation at $20000 \times g$, the pellet was resuspended in the above buffer to a chlorophyll concentration of 2 mg/ml.

Isolation of stroma lamellae

Thylakoids were disrupted by passing them three times through a French pressure cell operated at 1300 lb/in^2 with a flow rate of 8 ml/min. The suspension was then centrifuged for 30 min at $40000 \times g$ and the upper three-quarters of the supernatant was carefully transferred to ultracentrifuge tubes with a pipette, as described in Ref. 18. The stroma lamellae were pelleted at $110000 \times g$ for 30 min and resuspended in 200 mM sucrose/20 mM Hepes (pH 7.5)/ 5 mM

$MgCl_2$/10 mM NaF to a final chlorophyll concentration of 1.3 mg/ml.

Electrophoresis

SDS-PAGE was performed under non-denaturing conditions at 4°C as previously described [19,20] and under denaturing conditions in the presence of 6 M urea [21], and gels were fixed in methanol/ water/ acetic acid (2 : 2 : 0.4) and stained with Coomassie brilliant blue.

Isolation of LHC II, PS I-200 and PS II core particles

Stroma lamellae (1.0 mg Chl/ml) from light-adapted plants were washed in 5 mM Tricine (pH 8.0) and solubilised with 1.5% dodecyl maltoside (Calbiochem). Unsolubilised material was pelleted at 15000 × *g* for 10 min and the supernatant was layered over 12.5 ml of a 0.1 to 1.0 M sucrose gradient containing 1% dodecyl maltoside and 5 mM Tricine (pH 8.0) formed by freezing and slow thawing. Centrifugation at 39000 rpm for 17 h in a Beckman SW 41 rotor resulted in four separate bands, the upper one containing LHC II. PSI-200 was prepared by Triton X-100 solubilisation of destacked thylakoids, followed by sucrose gradient ultracentrifugation as described in Ref. 22. PS II core particles (CP48*) were prepared by octyl glucoside solubilisation of PS II membranes [23], followed by sucrose gradient ultracentrifugation [24].

Spectroscopy

Low-temperature fluorescence emission and excitation spectra were recorded wigh a Perkin-Elmer MPF44 spectrofluorimeter equipped with a low-temperature attachment. Samples were diluted to 5 μg Chl/ml, loaded into glass capillary tubes and frozen in liquid nitrogen. Spectra were not corrected. Chlorophyll concentration and *a/b* ratios were calculated from the optical absorbance in 80% acetone, measured to four decimal places in a Varian 2000 spectrophotometer, and corrected for any scattering by subtracting the absorbance at 700 nm. Absorption spectra were recorded with a Perkin-Elmer lambda 5 spectrophotometer, and circular dichroism (CD) spectra were recorded with a Jasco D-500 spectropolarimeter. P-700 concentration was measured as previously [22], and its photo-oxidation kinetics under light-limiting conditions (green light from a 550 nm interference filter at an intensity of 20 $\mu E/m^2$ per s) were measured by monitoring the absorbance decrease at 700 nm [25]. The Aminco DW2a spectrophotometer was operated in the dual beam mode, with the reference wavelength at 725 nm and side illumination. The photomultiplier tube was protected with a red Schott RG665 cut-off filter.

Frequency-domain lifetime measurements were made using the multifrequency phase and modulation fluorometer GREG-200 manufactured by ISS, La Spezia, Italy. This instrument, described elsewhere [26], operates using the cross-correlaiton principle introduced in Ref. 27. The light source in our application is a 150 W xenon lamp whose intensity is modulated with a Pockels cell. The modulation frequency is continuously variable from 1 to 190 MHz. Generally, a set of 8–12 different modulation frequencies were used in the range most appropriate to the sample under investigation. For each frequency the phase and modulation of the fluorescence were measured with respect to a scattering solution (glycogen). Data were collected by an IBM personal computer via a suitable interface supplied by ISS. The set of phase and modulation data was analysed by a non-linear least-squares routine described in Ref. 28. Data were fitted to a sum of exponential terms, each characterised by a lifetime, τ, and a fractional intensity, F. Pre-exponential factors can be obtained from the fractional intensities using the relation: $\alpha_i = (F_i/\tau_i)/(\Sigma_j F_j/\tau_j)$. The reduced χ^2) was used to judge the goodness of fit. Generally a value not greater than 3–4 was considered acceptable. In this work, any attempt to fit the exponential data with more than two components did not decrease the χ^2 value. This, of course, does not imply the existence of only two kinetic components, and demonstrates only that the model used is compatible with the data.

Freeze-fracture electron microscopy

Glycerol was added to unfixed stroma membranes to a final concentration of 20% and frozen and freeze-fractured with a Balzers BAF 301 freeze-etch apparatus at −110°C as previously described [29]. Replicas were examined with a Siemens Elmiskop 102 operated at 80 kV, and

calibrated at 50000 and 100000 magnification with a catalase grid (Balzers Union). Freeze-fracture particle densities were measured from electron micrographs enlarged to a final magnification of 200000 and 400000 as in Ref. 29. Only those areas of the fracture faces of vesicles which were flat enough to be uniformly shadowed were used for these measurements, to avoid overestimation of particle densities and a non-biased discrimination between large and small PF particles. By rejecting PG faces with a central unshaded area, the maximum error in particle density determination is $2/[(1+\sin(90°-9\theta)]$ ($=1.17$ when the shadowing angle $\theta = 22.5°$). The values obtained (Table I) are in good agreement with those for the EFu face of maize [30] and barley [29], while the PFu density is actually less (4308 vs. 4729 particles/μm^2 for barley). In any case, since the same criteria were applied to replicas of both dark- and light-adapted stroma lamellae, any error should be the same for both samples.

Labelling with ^{32}P

Isolated thylakoids were phosphorylated in the presence of [^{32}P]ATP and analysed by gel electrophoresis and autoradiography as previously described [31].

Results

The yield of stroma lamellae prepared by French press fractionation of maize thylakoids isolated from light- and dark-adapted leaves was similar (i.e., 3.8% and 4.0%, respectively, of the total chlorophyll). Freeze-fracture electron microscopy revealed that the preparations consisted largely of spherical, right-side-out vesicles (Fig. 1). The pitted appearance of the EF face, together with the low particle density, was characteristic of the EF face of the stroma membranes from which these vesicles were derived [29]. Contamination by appressed grana partition membranes, which were easily recognised by their high EF particle density,

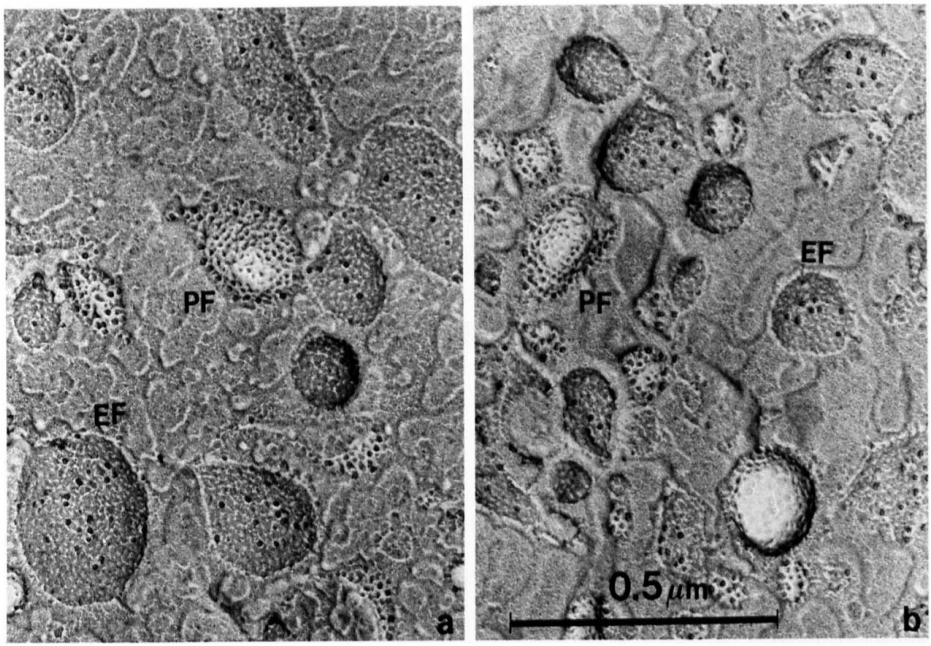


Fig. 1. Freeze-fracture electron microscopy of stroma membrane vesicles from (a) dark-adapted plants and (b) light-adapted plants. Note the characteristic appearance of the convex protoplasmic fracture face (PF) as well as the concave endoplasmic fracture faces (EF). Magnification 92400×.

TABLE I

FREEZE-FRACTURE PARTICLE DENSITIES.

Values are particles per $\mu m^2 \pm$ S.D.

Frace	Dark-adapted stroma lamellae	Light-adapted stroma lamellae
EF	407 ± 23	480 ± 27
PF	4308 ± 50	4523 ± 57
PG large	3060 ± 52	3097 ± 59
PF small	1248	1426

was exceptionally low, being of the order of 0.1% in both preparations. Analyses of the particles on the EF and PF faces revealed an 18% increase in EF particle density, and a slight increase in PF particle density, due mainly to an increase in the small PF particles (Table I). The two stroma preparations differed significantly in their chlorophyll *a/b* ratios, 8.4 for stroma lamellae isolated from dark-adapted leaves compared with 6.2 for light-adapted leaves. This increase in the level of chlorophyll *b* in stroma lamellae indicates a transfer of chlorophyll *b*-rich chlorophyll-*a/b*-containing complexes from grana to stroma labellae as a result of the state 1 to state 2 transition in vivo.

In principle, an alteration in excitation energy distribution in response to adaptation to light could be effected by a decrease in the PS II antenna size by disconnection of some of the LHC II. But this does not require lateral movement of LHC II from the grana, with a consequent decrease in the chlorophyll *a/b* ratio of stroma lamellae, as found above. An increase in PS I antenna size would mean that the chlorophyll-*a/b*-proteins that migrated into the stroma were able to transfer excitation energy to the PS I reaction centre. Measurement of the chlorophyll: P-700 ratios for dark- and light-adapted stroma lamellae showed an increase from 160 to 184 (Table II). This was accompanied by a corresponding decrease in the time constant ($t_{1/2}$) for P-700 photo-oxidation (Fig. 2) determined under conditions where the light intensity was limiting, and at a wavelength where chlorophylls *a* and *b* absorb equally. Both measurements are consistent with an approx. 15% increase in PSI antenna size in light-adapted stroma lamellae.

TABLE II

PROPERTIES OF ISOLATED MAIZE STROMA LAMELLAE

Sample	Chl *a/b*	Chl/P-700 [a]	$t_{1/2}$ [a]
Dark-adapted	8.4	160 ± 7	0.775 ± 0.003
Light-adapted	6.2	184 ± 6	0.630 ± 0.002

[a] Values are ± S.D. and are the average of at least four determinations.

To characterise the nature of the chlorophyll-*a/b*-protein which moved into the stroma in response to light, we analysed the chlorophyll-protein composition of stroma isolated from dark- and light-adapted leaves. Mild SDS-PAGE of stroma lamellae (Fig. 3) resolved several different chlorophyll-protein complexes: two P-700-containing complexes (bands 1a and 1b), two or three LHC I-730 bands (bands 2, 3a and 3b) and one LHC I-680 band (band 4b). A small amount of LHC II formed two bands in the gel – an oligomeric form co-migrating with an oligomeric form of LHCI-730 (band 2) and a faster moving momomeric form (band 4a). The identity of these bands was verified by analysis of their polypeptide composition by denaturing SDS-PAGE. Other chlorophyll-protein bands, including CP47, CP43 and CP29 as well as other PS II antenna complexes [24,31] were not detected. Densitometric

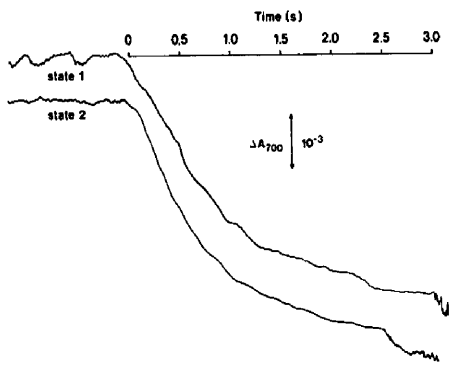


Fig. 2. Time-course of P-700 oxidation in stroma lamellae incubated with 0.5 mM ascorbate and side-illuminated with non-saturating 550 nm light. The spectrophotometer was operated in the duel beam mode with the reference wavelength at 725 nm.

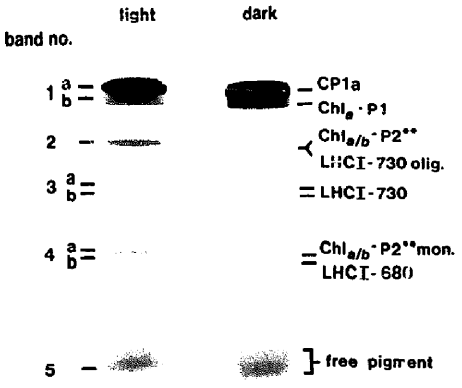


Fig. 3. Separation of chlorophyll-proteins by non-denaturing SDS-PAGE of stroma membranes isolated from dark- and light-adapted maize leaves. Membranes were solubilised with the non-ionic detergent octyl glucoside (detergent/chlorophyll = 20:1) and gels run at 4°C for 3 h at 0.4 mA per tube. The same amount of chlorophyll (20 μg) was loaded in each tube.

scans (not shown) of the gels revealed that only bands 2 and 4a (i.e., those containing LHC II) showed a relative increase in light-adapted stroma.

The polypeptide patterns of stroma lamellae isolated from dark- and light-adapted plants is shown in Fig. 4 with those of thylakoids and grana partition membranes for comparison. It was clear that the polypeptide compositions of stroma and grana membranes were mutually exclusive, further confirming the purity of the stroma preparations. The only difference between the two stroma membrane preparations was in the 28–30 kDa range, where there was a significant increase in the amount of the LHC II polypeptides in the light-adapted stroma lamellae (Fig. 4). The increase in the level of LHC II in light-adapted stroma was

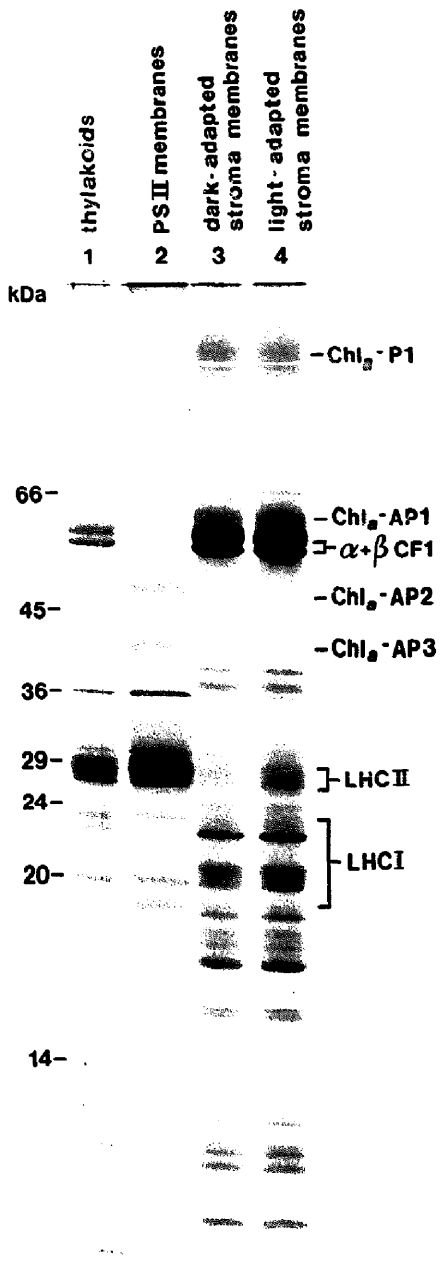
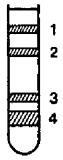


Fig. 4. Polypeptide composition of dark- and light-adapted stroma lamellae revealed by denaturing SDS-PAGE in the presence of 6 M urea. The same amount of chlorophyll was loaded in each lane, and the positions of molecular mass markers are shown in kDa. Note the differences in composition between PS II and stroma membranes.



1% dodecylmaltoside
27hrs.39.000rpm in SW40 rotor

Fig. 5. Diagrammatic representation of the fractionation pattern of light-adapted stroma membranes after sucrose-dodecyl maltoside gradient ultracentrifugation. The position of the green bands are indicated. The uppermost band (1) was highly fluorescent.

confirmed by polypeptide analysis (not shown) of the chlorophyll-proteins in bands 2 and 4a (Fig. 3).

Stroma membranes were solubilised with dodecyl maltoside, followed by sucrose gradient centrifugation, as described in Material and Methods. Four chlorophyll-containing bands were obtained (Fig. 5), the uppermost one consisting of almost pure LHC II (Fig. 6a). Gel scans of the polypeptide composition of the LHC II isolated from light-adapted stroma lamellae and grana lamella (Fig. 6b) showed that both contained bands with apparent molecular masses of 28.5, 29, 29.5 and 30 kDa. The stroma lamellae LHC II was enriched in the lower-mass bands, with an approximate 1 : 1 : 1 : 1 stoichiometry, and lacked the 26 kDa band. The sucrose gradient LHC II bands from light-adapted stroma membranes was analysed by absorption, low-temperature fluorescence and circular dichroism spectroscopy, and compared with LHC II from grana membranes (Fig. 7a,b). The small differences found indicated that the LHC II which moved out of the grana (mobile LHC II) and that which stayed in grana (non-mobile LHC II) were similar but distinct chlorophyll-proteins.

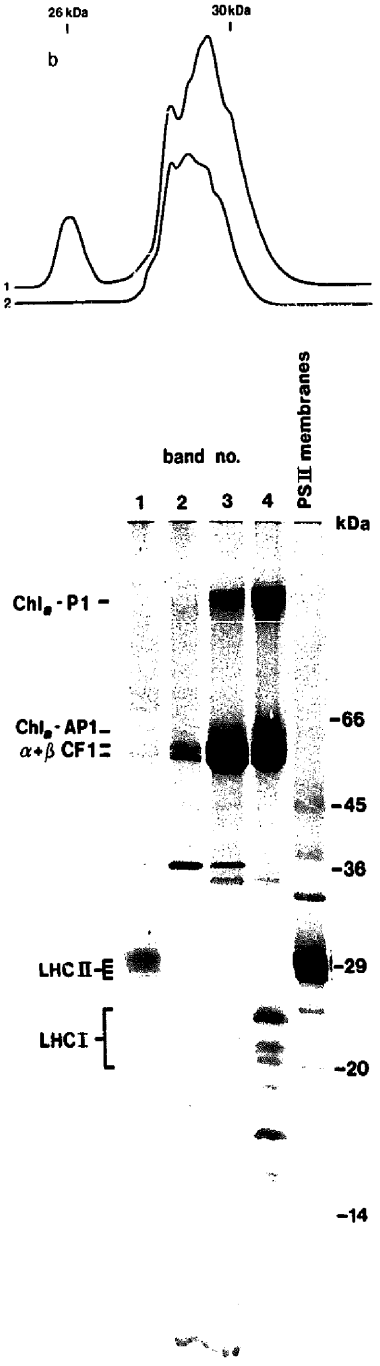


Fig. 6. (a) Polypeptide composition of the four chlorohyll-containing bands separated from light-adapted stroma membrane in Fig. 5. Band 1 is almost pure LHC II, consisting of only four polypeptide components. (b) Gel scan of the polypeptides of LHC II isolated from (1) grana lamellae and (2) light-adapted stroma lamellae. The 26 kDa polypeptide is absent from the latter, and the stoichiometry of the other four bands is different from that of LHC II from grana lamellae.

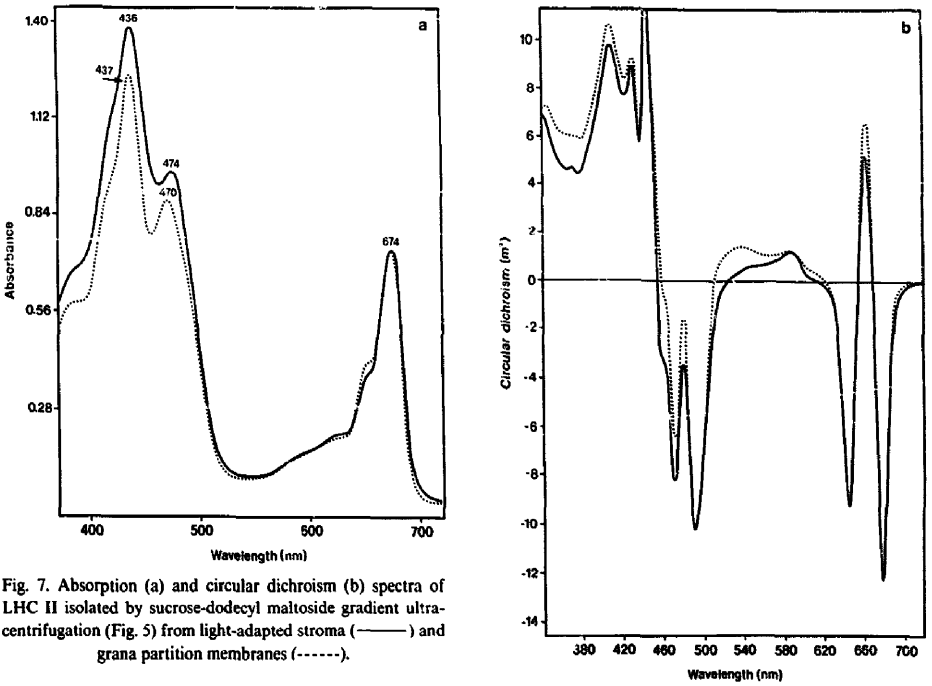


Fig. 7. Absorption (a) and circular dichroism (b) spectra of LHC II isolated by sucrose-dodecyl maltoside gradient ultracentrifugation (Fig. 5) from light-adapted stroma (———) and grana partition membranes (------).

Functional differences between these two types of LHC II were further investigated by phosphorylation experiments. Isolated thylakoid membranes were incubated with [^{32}P]ATP under suitable illumination conditions, their polypeptides were separated by denaturing SDS-PAGE, and phosphorylated protein were detected by autoradiography. The resulting nine labelled bands were compared with the polypeptides in Coomassie-stained preparations of thylakoids and the PS II reaction centre core (Fig. 8). Most of the label was concentrated in LHCII, but not the 26 kDa polypeptide. Three other labelled bands co-migrated with PS II core polypeptides, namely the 43 kDa chlorophyll-*a*-binding protein (CP43), the 34 kDa (D1) and the 32 kDa (D2) polypeptide. A 9 kDa phosphoprotein was observed with the same mobility as the cytochrome *b*-559 apoprotein, but is perhaps more likely to correspond with the 9–10 kDa phosphoprotein associated with PS II [32]. A faint band was also detected having a mobility of 55 kDa, which may be the D1/D2 heterodimer described by Marder et al. [33].

Further investigation of the distribution of excitation energy in dark- and light-adapted stroma labellae was made by fluorescence spectroscopy. The low-temperature (77 K) fluorescence emission spectrum of whole thylakoids has three well-resolved peaks, centered at 685 nm, 695 nm and 735 nm, and these have been assigned to LHC II, PS II and PS I, respectively [34,35]. The isolated stroma lamellae showed a single major peak at 732 nm (see Fig. 9, control), in agreement with previous studies [18], for both dark- and light-adapted plants. We studied the effect on the fluorescence emission spectra of light-adapted stroma lamellae of increasing concentrations of the detergent octyl glucoside, which has previously been shown to disconnect LHC II, but not LHC I, from the PS I reaciton centre at low concentrations [21]. The stroma lamellae showed the typical results of antenna disconnection, with an increase in fluores-

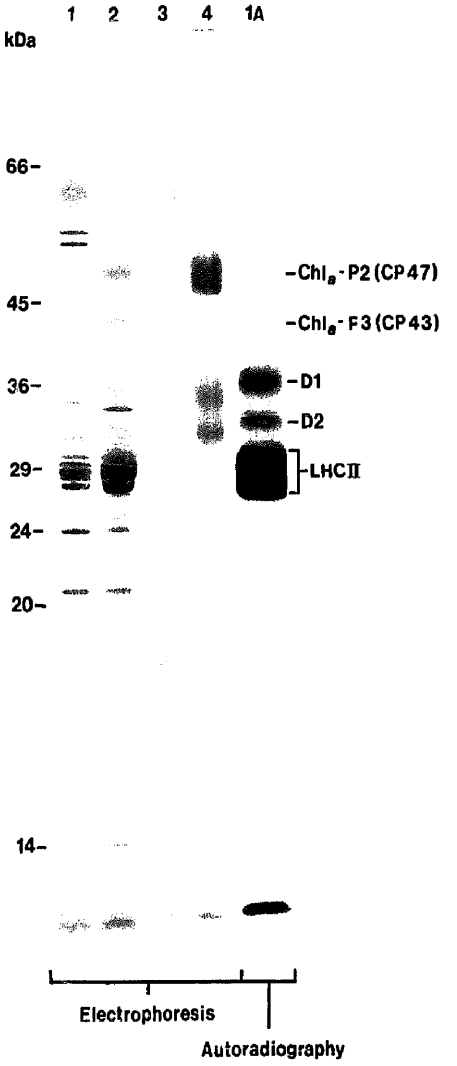


Fig. 8. Polypeptide composition of thylakoids (lane 1) and subthylakoid fractions (lane 2 = PS II membranes; lane 3 = PS I-200; lane 4 = PS II core particles) separated by denaturing SDS-PAGE in the presence of 6 M urea. Lane 1A is an autoradiograph of thylakoids labelled with [γ-^{32}P]ATP.

cence field at 678 nm, and a decrease at 732 nm (Fig. 9), which we attribute to the disconnection of the highly fluorescent LHC II. Dark-adapted stroma lamellae showed similar but smaller changes in their 77 K fluorescence emission spectrum due to the presence of a low level of LHC II. Fluorescence excitation spectroscopy confirmed that excitation of chlorophyll *b* (at 468 nm) produced a relatively higher emission at 732 nm in light-adapted stroma compared with dark-adapted stroma (Fig. 10). Addition of increasing amounts of octyl glucoside (0.05 to 0.4%) caused a progressive decrease in the 468 nm peak of the excitation spectrum relative to the 435 nm peak (Fig. 10), consistent with a decrease in the amount of chlorophyll *b* transferring excitation energy to PS I. Addition of up to 0.4% octyl glucoside to dark-adapted stroma lamellae caused a similar decrease

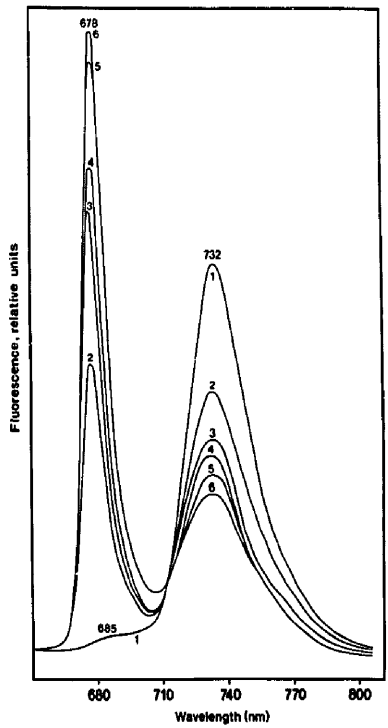


Fig. 9. Low-temperature fluorescence (77 K) emission spectra of stroma membranes isolated from light-adapted leaves in the presence of varying amounts of octyl glucoside. The membranes were diluted in 5 mM Tricine-NaOH (pH 8.0) at a chlorophyll concentration of 5 μg/ml and incubated for 5 min on ice at the desired detergent concentration before freezing. (1) Control; (2) 0.05% octyl glucoside; (3) 0.1%; (4) 0.2%; (5) 0.3%; (6) 0.4%. Excitation was at 445 nm (6 nm slit), with an emission slit of 3 nm. Note particularly the absence of a 685 nm peak, in spite of the presence of LHC II.

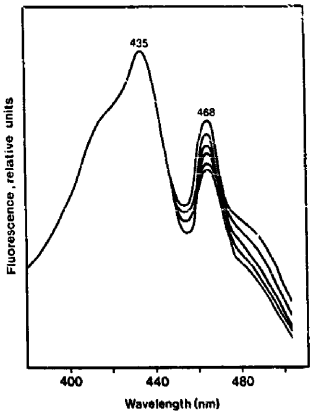


Fig. 10. Low-temperature (77 K) fluorescence excitation spectra of light-adapted stroma membranes exposed to increasing concentrations of octyl glucoside (0, 0.05, 0.1, 0.2, 0.3, 0.4%) recorded at 732 nm. Spectra have been normalised at 435 nm, and show a reduction in the contribution of chlorophyll *b* (at 468 nm) to 732 nm fluorescence emission as increasing concentrations of octyl glucoside disconnect more LHC II from PS I.

in the 468 nm peak, but was only about one-quarter of the amplitude of the change seen in light-adapted stroma lamellae.

The results of the fluorescence lifetime measurements are summarised in Table III. Within the limits of precision, the phase and modulation data were best fitted by two lifetime components for stroma lamellae, and a single lifetime component for LHC II. The determination of fluorescence lifetimes for stroma lamellae in the absence of detergent was particularly difficult due to the very high quenching activity of the PS I reaction centre. No significant difference could be found between light- and dark-adapted samples. In both samples, the long lifetime (approx. 2 ns) component was very small (under 5%) and most of the fluorescence was emitted with a lifetime of 300 ps or less (small fractions of faster decaying components, less than 10 ps, could not be detected with our present apparatus). In the samples treated with octyl glucoside, the amount of fluorescence emitted with longer lifetimes increased to 6% in the dark-adapted stroma, and no 11% in the light-adapted stroma lamellae (Table III).

We interpret the 2 ns fluorescence component as coming from LHC II disconnected from the PS I reaction centre, whereas the 300 ps emission originates from chlorophyll closer to the PS I reaction centre, possibly from LHC I. This is supported by the increase in fluorescence lifetime of isolated LHC II, from 685 ps to 3.7 ns as a result of detergent-induced disaggregation (Table III). A similar increase from 816 ps to 3.1 ns has been reported in Ref. 36. The differences between

TABLE III

FLUORESCENCE LIFETIME DATA

Excitation was at 430 ± 5 nm, emission wavelengths higher than 550 nm were analysed. Chlorophyll concentration was 17.5 μg/ml, temperature 10 °C. Where indicated, samples are treated with 0.5% octyl glucoside. LHC II was prepared by Mg^{2+} precipitation of the sucrose gradient band from Triton X-100 solubilised thylakoids using the procedure of Ref. 55. $Chl_{a/b}$-P2 ** is the oligomeric form of LHC II, excised from SDS-PAGE gels. LHC II from stroma is band 1 in fig. 6. PS II membranes were prepared according to Ref. 23. F_1 is the fraction of the integrated fluorescence emission decaying with τ_1. α_1 is the molar fraction of the species emitting with τ_1. χ^2 represents the goodness of fit to the data. Values are ± S.D.

Sample	τ_1 (ps)	F_1	α_1	τ_2 (ps)	χ^2
LHC II	665 ± 4	1.0	1.0	–	2.15
LHC II + detergent	3680 ± 20	1.0	1.0	–	5.8
$Chl_{a/b}$-P2 **	2890 ± 0.1	0.66	0.18	375 ± 0.03	2.2
LHC II from stroma	3766 ± 0.02	1.0	1.0	–	3.2
PS II membranes	435 ± 0.01	1.0	1.0	–	2.1
PS II membranes + detergent	645 ± 0.04	0.71	0.93	3613	3.3
Dark stroma	1800 ± 200	0.10	0.02	270 ± 70	3.4
Light stroma	1400 ± 100	0.17	0.04	300 ± 80	1.4
Dark stroma + detergent	1800 ± 400	0.29	0.06	300 ± 50	2.17
Light stroma + detergent	1800 ± 400	0.38	0.11	360 ± 80	2.1

the lifetimes of isolated LHC II treated with octyl glucoside, and mobile LHC II (3.7 vs. 1.8 ns) may be attributed to differences in their structural organisation. In fact, LHC II isolated from light-adapted stroma lamellae also had a lifetime of 3.8 ns, whereas LHC II isolated from gels (i.e., $Chl_{a/b}$-P2 **) had a lifetime of 2.9 ns. A component with a single fluorescence decay lifetime of 2.6 ns has been attributed to disconnected antenna molecules in a *Chlamydomonas* mutant lacking PS I and PS II [37].

Discussion

In the proposed model for chloroplast state transitions in higher plants [10], a central role is played by a LHC II population which upon phosphorylation detaches from PS II in grana partitions [38] and migrates into the stroma membranes [15,39,40], where it may transfer energy to PS I. However, much of the work concerning the role of LHC II phosphorylation in regulating energy distribution between PS I and PS II has been done with in vitro systems, utilising isolated chloroplasts or thylakoids. The in vivo studies have yielded contrasting results. When intact cells of Chlamydomonas were incubated with [^{32}P]orthophosphate, no differences were observed between dark- and light-adapted cells [41]. No correlation was found between state transitions and LHC II phosphorylation in wheat, and the addition of zinc ions was found to stimulate LHC II phosphorylation, while inhibiting state transitions [42]. Heat treatment was reported to induce state 2 in the absence of ATP [43]. On the other hand, the phosphorylation of specific polypeptides has been correlated with the induction of the state 1–state 2 transition, both in vitro and in vivo [44], and immersion of leaves in a solution containing NaF, which inhibits the phosphatase, abolished the state 2–state 1 transition [45].

In this paper, we have isolated stroma lamellae which have a low level of contamination by grana membranes. This high purity is achieved at the expense of yield (4% of total chlorophyll versus a theoretical maximum of 33%), but we regard these preparations as representative of stroma lamellae based on their freeze-fracture ultrastructure and polypeptide composition. There is no major grana component which is completely absent from stroma lamellae and this, together with the possible sensitivity of PS II-dependent oxygen evolution to damage during the isolation procedure, makes it difficult, using these criteria, to rule out grana contamination below the 1–5% level [18]. The use of freeze-fracture electron microscopy to identify grana partition membranes by their distinctive appearance overcomes these problems, and confirms the functional and compositional measurements. An examination of over 3000 stroma lamellar vesicles in each preparation revealed only 2 or 3 grana-derived vesicles, giving an upper estimate of contamination of about 0.1%.

The state 1–state 2 transition produces an increase in the chlorophyll/P-700 ratio from 160 to 184. Analysis by freeze-fracture electron microscopy showed that this was not due to increased contamination by appressed grana membranes, and a lateral migration of PS II into stroma lamellae can also be excluded, since Mg^{2+} was always present at 10 mM, and the fluorescence yield at 685–695 nm (due to PS II) remained consistently low. In fact, the kinetics of P-700 photo-oxidation under light-limiting condi-

TABLE IV

DISTRIBUTION OF CHLOROPHYLL MOLECULES IN PS I BASED ON THE MODEL OF REF. 22

	Chl *a*	Chl *b*	Dark-adapted		Light-adapted	
			Cgl/P-700	Chl *a*/*b*	Chl/P-700	Chl *a*/*b*
Chl_a-P1	92	0	160	8.4	181	6.0
LHC I	51	17				
LHC II [a]	12	9				

[a] Chl *a*/*b* = 1.33 * [56].

tions showed that the increased chlorophyll/P-700 ratio was due to an increase in antenna size. The chlorophyll distribution between the different chlorophyll-proteins of PS I can be calculated using the model of Ref. 22 and the chlorophyll *a/b* and chlorophyll/P-700 values (Table IV). This shows that approx. 21–24 chlorophylls as LHC II become functionally attached to the PS I reaction centre. This corresponds to an approximate increase of 15% in PS I antenna size, which is consistent with the decrease in variable fluorescence associated with the state 1–state 2 transition [46,47], and a 17% increase in PS I antenna size induced in vitro in isolated thylakoids [48], measured by the kinetics of non-saturating laser flash-induced absorption change at 820 nm. Our results do not agree with those of Haworth and Melis [49], who found no change in PS I antenna size after LHC II phosphorylation in isolated thylakoids, within the 10% error of their measurements. Our use of isolated stroma vesicles, which are 3-times enriched in P-700 with respect to chlorophyll, when compared to whole thylakoids, may account for this difference. In *Chlamydomonas* cells [40], the redox state of the plastoquinone pool can be manipulated through the chlororespiratory pathway to produce a 50% decrease in variable fluorescence, with a 50% increase in PS I antenna size [50]. Similarly, a 45% increase in PS I antenna size was found in vitro in destacked thylakoids [48]. This corresponds to about 100 molecules of chlorophyll as LHC II, which we have previously shown is the maximum that can functionally attach to PS I in vitro [22]. Since trypsin cleavage of the eight N-terminal amino acids of LHC II (containing the site of phosphorylation) does not disconnect LHC II from PS I in vitro [22], phopshorylation of LHC II (and other PS II polypeptides) in vivo may be required for its disconnection from PS II, rather than connection to PS I.

The state 1–state 2 transition was accompanied by an 18% increase in EF particle density in isolated stroma lamellae. This corresponds to a calculated change in stacking of 4% (from 67% to 63%), consistent with previous results with barley [51], and with the 10% found by others [48,52]. A small increase in PF particle density (Table III) is largely due to an increase in small particles (178 per μm^2). The question arises as to whether these extra small PF particles correspond to the LHC II which has moved from grana to stroma lamellae. It is generally agreed that each EF particle in grana membranes contains one PS II reaction centre, and the PF particles correspond to LHC II, there being about 4 PFs particles per EFs particle [29]. Since there are about 250 chlorophyll molecules per PS II reaction centre in appressed grana lamellae, and 50 chlorophyll *a* molecules per PS II core [25], we can estimate that each PFs particle contains at most 50 molecules of chlorophyll as LHC II. The increase in PS I antenna size as a result of the state 1–state 2 transition, therefore requires the transfer of 1 PFs particle per 2 PS I reaction centres, which correspond to the large PF particles in the stroma lamellae [53]. Based on the assumptions above, this can be accomplished by a 9% decrease in the extent of stacking (e.g., from 67% to 58%, see also Ref. 48), given that PFs particle density does not change significantly after phosphorylation [39,51]. However, the expected increase in small PF particle density as a result of LHC II migration (i.e., an extra 1530 particles per μm^2) is not observed (Table III and Ref. 51). It is probable, therefore, that the phosphorylated LHC II associated with PS I forms part of the large PF particles.

We verified that the LHC II was able to transfer excitation energy to PS I by fluorescence emission, fluorescence excitation and fluorescence lifetime measurements. The characteristic properties of non-associated LHC II (fluorescence emission at 685 nm with a lifetime of 2 ns) was seen only when light-adapted stroma vesicles were treated with the detergent octyl glucoside, which dissociates LHC II from PS I [21,22]. Fluorescence excitation spectroscopy showed a greater contribution of chlorophyll *b* to the 730 nm fluorescence in light-adapted stroma compared with dark-adapted stroma, again confirming the increase in the amount of chlorophyll *b* in the PS I antenna.

Our results extend previous reports [54] of a different level of phosphorylation of spinach LHC II polypeptides (25 and 27 kDa) and an enrichment of the more heavily labelled component (25 kDa) in the stroma lamellae. We have been able to resolve maize mesophyll LHC II, defined as the chlorophyll-protein complex which forms an

oligomer in SDS-PAGE with an apparent molecular mass of 64 kDa (band 2 in Fig. 3), into five separate Coomassie-blue-staining bands. Up to four of these polypeptides become phosphorylated (Fig. 8) and move from grana to stroma lamellae, possibly in a complex in which these polypeptides are present in a ratio of approx. 1:1:1:1. The fifth polypeptide (26 kDa) does not become phosphorylated, and remains in the appressed regions of the grana. The different stoichiometry of the polypeptide components of mobile LHC II and the LHC II of the grana would suggest that the organisation of LHC II is complex. One type of LHC II contains only four different polypeptides, as far as one can tell from SDS-PAGE, and these undergo lateral migration upon phosphorylation. Other LHC II complexes have a different composition and may or may not include the 26 kDa polypeptide. These are more tightly bound to PS II and may or may not become phosphorylated.

We have considered the following questions: (i) is the state 1–state 2 transition accompanied by migration of LHC II from grana to stroma lamellae, as occurs in vitro? (ii) if so, does it transfer excitation energy to PS I reaction centres? and (iii) what is the polypeptide composition of such a 'mobile' LHC II?. The problem has been approached by isolating stroma lamellae from maize plants adapted in the dark (state 1) or to high-intensity white light (state 2).

We have shown that the state 1–state 2 transition results in the movement of LHC II from the appressed membranes of grana regions to the stroma lamellae. The chlorophyll-*b*-rich LHC II increases the chlorophyll *b* content of stroma lamellae, with a consequent decrease in the chlorophyll *a*/*b* ratio. The LHC II becomes functionally associated with PS I reaction centres and consists of four polypeptides in an approx. 1:1:1:1 stoichiometry, all of which become phosphorylated. A 26 kDa polypeptide which is found in LHC II isolated from grana partition membranes is not a component of the mobile LHC II and is not phosphorylated in vitro.

Acknowledgements

We thank Professor Diter von Wettstein for his encouragement during the course of this work, and for critically reading the manuscript. We are grateful to Professor Birger Lindberg Møller for discussions on the isolation of stroma lamellae. We thank Nina Rasmussen for drawing the figures, and Ann-Sofi Steinholtz for printing the photographs.

References

1 Björkman, O. and Ludlow, M.M. (1972) Carnegie Inst. Wash. Yearb. 71, 85–94.
2 Björkman, O. (1981) in Encyclopedia of Plant Physiology (Lange, O.K., Nobel, P.A., Osmond, C.B. and Zeigler, H., eds.), Vol. 12A, pp. 57–107, Springer Berlin.
3 Bonaventura, C. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366–383.
4 Chow, W.S., Telfer, A., Chapman, D.J. and Barber, J. (1981) Biochim. Biophys. Acta 638, 60–68.
5 Murata, N. (1969) Biochim. Biophys. Acta 189, 171–181.
6 Barber, J. (1982) Annu. Rev. Plant Physiol. 37, 93–136.
7 Bennett, J. (1979) Eur. J. Biochem. 99, 133–137.
8 Allen, J.F., Steinback, K.E. and Arntzen, C.J. (1981) Nature 291, 25–29.
9 Haworth, P., Kyle, D.J., Horton, P. and Arntzen, A. (1982) Photochem. Photobiol. 36, 743–748.
10 Staehelin, L.A. and Arntzen, C.J. (1983) J. Cell Biol. 97, 1327–1337.
11 Bennett, J. (1977) Nature 269, 344–346.
12 Coughlin, S.J. and Hind, G. (1986) J. Biol. Chem. 261, 11378–11385.
13 Horton, P. and Black, M.T. (1980) FEBS Lett. 119, 141–144.
14 Telfer, A. and Barber, J. (1981) FEBS Lett. 129, 161–164.
15 Andersson, B., Åkerlund, H.-E., Jergil, B. and Larsson, C. (1982) FEBS Lett. 149, 181–184.
16 Kannangara, C.G., Gough, S.P., Hansen, B., Rasmussen, J.N. and Simpson, D.J. (1977) Carlsberg Res. Commun. 42, 431–439.
17 Bassi, R. and Simpson, D.J. (1986) Carlsberg Res. Commun. 51, 363°370.
18 JHenry, L.E.A. and Møller, B.L. (1981) Carlsberg Res. Commun. 46, 183–194.
19 Bassi, R., Dal Belin Peruffo, A., Barbato, R. and Ghisi, R. (1985) Eur. J. Biochem. 146, 589–595.
20 Bassi, R., Machold, O. and Simpson, D.J. (1985) Carlsberg Res. Commun. 50, 145–162.
21 Bassi, R. (1985) Carlsberg Res. Commun. 50, 127–143.
22 Bassi, R. and Simpson, D.J. (1987) Eur. J. Biochem. 163, 221–230.
23 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
24 Bassi, R., Høyer-Hansen, G., Barbato, R., Giacometti, G.M. and Simpson, D.J. (1987) J. Biol. Chem. 262, 13333–13341.
25 Melis, A. and Anderson, J.M. (1983) Biochim. Biophys. Acta 724, 473–484.
26 Gratton, E. and Limkeman, M. (1983) Biophys. J. 22, 315–324.

27 Spencer, R.D. and Weber, G. (1969) Ann. N.Y. Acad. Sci. 158, 361–376.
28 Lakowicz, J.R., Kherek, H., Maliwal, B.P. and Gratton, E. (1985) Biochemistry 24, 376–383.
29 Simpson, D.J. (1979) Carlsberg Res. Commun. 44, 305–336.
30 Miller, K.R., Miller, G.J. and McIntyre, K.R. (1977) Biochim. Biophys. Acta 459, 145–156.
31 Bassi, R. and Simpson, D.J. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 2, 81–88, Martinus Nijhoff, Dordrecht.
32 Farchaus, J. and Dilley, R.A. (1985) Arch. Biochem. Biophys. 244, 94–101.
33 Marder, J.B., chapman, D.J., Telfer, A., Nixon, P.J. and Barber, J. (1987) Plant Mol. Biol. 9, 325–333.
34 Rijsberger, C.P., Amesz, J., Thielen, A.P. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482.
35 Moya, I. and García, R. (1983) Biochim. Biophys. Acta 722, 480–491.
36 Hodges, M., Moya, I., Briantais, J.-M. and Rémy, R. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 1, 115–118, Martinus Nijhoff, Dordrecht.
37 Gulotty, R.J., Mets, L., Alberte, R.A. and Fleming, G.R. (1985) Photochem. Photobiol. 41, 487–496.
38 Torti, F., Gerola, P.D. and Jennings, R.C. (1984) Biochim. Biophys. Acta 680, 352–360.
39 Kyle, D.J., Staehelin, L.A. and Arntzen, C.J. (1983) Arch. Biochem. Biophys. 222, 527–541.
40 Kyle, D.J., Kuang, T.Y., Watson, J.L. and Arntzen, C.J. (1984) Biochim. Biophys. Acta 765, 89–96.
41 Owens, G.C. and Ohad, I. (1982) J. Cell. Biol. 93, 712–718.
42 Baker, N.R., Markwell, J.P., Webber, A.N. and Thornber, J.P. (1984) in Advances Photosynthesis Research (Sybesma, C., ed.), Vol. 3, 319–322, Martinus Nijhof/Junk, Dordrecht.
43 Sane, P.V., Desai, T.S., Tatake, V.G. and Govindjee (1984) Photosynthetica 18, 439–444.
44 Wollman, F.-A. and Delepelaire, P. (1984) J. Cell Biol. 98, 1–7.
45 Canaani, O., Barber, J. and Malkin, S. (1984) Proc. Natl. Acad. Sci. USA 81, 1614–1618.
46 Farchaus, J.W., Widger, W.R. Cramer, W.A. and Dilley, R.A. (1982) Arch. Biochem. Biophys. 217, 363–367.
47 Telfer, A., Hodges, M., Millner, P.A. and Barber, J. (1984) Biochim. Biophys. Acta 766, 554–562.
48 Telfer, A., Bottin, H., Barber, J. and Mathis, P. (1984) Biochim. Biophys. Acta 764, 324–330.
49 Howarth, P. and Melis, A. (1983) FEBS Lett. 160, 277–280.
50 Delepelaire, P. and Wollman, F.-A. (1985) Biochim. Biophys. Acta 809, 277–283.
51 Simpson, D.J. (1983) Biochim. Biophys. Acta 725, 113–120.
52 Biggins, J. (1982) Biochim. Biophys. Acta 679, 479–482.
53 Simpson, D.J. (1983) Eur. J. Cell Biol. 31, 305–314.
54 Larsson, U.K. and Andersson, B. (1985) Biochim. Biophys. Acta 809, 396–402.
55 Burke, J.J., Ditto, C.I., and Arntzen, C.J. (1979) Arch. Biochem. Biophys. 187, 252–263.
56 Hinz, U.G. and Welinder, K.G. (1987) Carlsberg Res. Commun. 52, 39–54.