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Photosystem stoichiometry and chlorophyll antenna size in *Dunaliella salina* (green algae)

J.E. Guenther, J.A. Nemson and A. Melis

Division of Molecular Plant Biology, University of California, Berkeley, CA (U.S.A.)

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Key words: Photosystem stoichiometry; Photosynthetic unit size; Light harvesting complex; Photosystem II heterogeneity; (*D. salina*)

The photochemical apparatus organization in the green alga *Dunaliella salina* was investigated. Photosystem (PS) stoichiometry was estimated from direct quantitations of the reaction center P-700 (PS I) and of the primary electron acceptors pheophytin and Q_A (PS II). A PS II/PS I ratio of 1.4/1.0 was deduced. Analysis of PS II activity revealed heterogeneity both in the chlorophyll (Chl) antenna size of the PS II unit and in the electron-transport process from Q_A to plastoquinone in the thylakoid membrane. Antenna size measurements indicated the presence of PS II_α (55% of all PS II) with about 570 Chl ($a + b$) molecules, and of PS II_β (45% of all PS II) with about 150 Chl ($a + b$) molecules per reaction center. By comparison, PS I contained about 220 Chl ($a + b$) molecules per reaction center. Analysis of the electron-transport reactions on the reducing side of PS II revealed that approximately 25% of all PS II centers, although photochemically competent, were unable to transfer electrons from the primary quinone acceptor Q_A to the secondary quinone Q_B (PS II-Q_B-nonreducing). Based on the fluorescence yield and exponential induction kinetics displayed by PS II-Q_B-nonreducing, we suggest that the latter is a subpopulation of PS II_β in *Dunaliella salina*. It is speculated that PS II-Q_B-nonreducing centers represent newly synthesized and/or repaired PS II centers which have not yet established a functional interaction between Q_A and Q_B.

Introduction

Research over the last several years has yielded important information on the organization, structure and function of the components in the thylakoid membrane of photosynthesis. In higher plant chloroplasts, the thylakoid membrane is divided into two distinct structural entities, the grana with appressed thylakoid membranes, and the stroma-exposed lamellae [1]. It has been recognized that such structural differentiation facilitates a functional differentiation in the thylakoid membrane. It has been shown that 70–80% of all PS II complexes (PS II_α) are localized in the membrane of the grana partition region, whereas PS I complexes and the remainder of PS II (PS II_β, about 20–30% of total PS II) are localized exclusively in the intergrana and stroma-exposed lamellae [2–4]. A direct consequence of the lateral separation of the two photosystems in the thylakoid membrane is the realization that each photosystem is a structurally and functionally distinct entity possessing its own Chl *a*-core and Chl *ab* accessory light-harvesting (LHC) antenna [5–7].

Abbreviations: Chl, chlorophyll; PS, Photosystem; Q_A, primary quinone acceptor of PS II; Q_B, secondary quinone acceptor of PS II; P-700, reaction center of PS I; Pheo, pheophytin; DCMU, dichlorophenyldimethylurea; LHC, light-harvesting complex; PS II-Q_B-nonreducing, PS II center with impaired Q_A–Q_B interaction; PAGE, polyacrylamide gel electrophoresis.

Correspondence: A. Melis, Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720, U.S.A.

An independent development concerning the structural and functional organization of the chloroplast photosystems was the discovery of a markedly variable stoichiometry of PS I and PS II among different photosynthetic tissues [8]. This understanding became possible upon the application of direct spectrophotometric techniques in the quantitation of photosystem stoichiometry in chloroplasts. In specific examples of photosystem stoichiometry ratios, it was reported that cyanobacteria commonly display PS II/PS I reaction center ratios well below unity (PS II/PS I = 0.4 [8–10]) while in higher plant chloroplasts like spinach, PS II/PS I = 1.8 [8,11]. Mutants deficient in the Chl *ab* light-harvesting complex displayed PS II/PS I ratios greater than 2 : 1 [12–14].

Another group of photosynthetic organisms, the *Chlorophyta* or green algae, possess a photochemical apparatus that in many respects resembles that of higher plant chloroplasts, i.e., the presence of Chl *b* and the appearance of appressed thylakoids. However, a clear differentiation of the thylakoid membrane into grana stacks and interconnecting stroma thylakoids is missing. Green algae display a thylakoid lamella system composed of extensive membrane bands, each containing 2–6 appressed thylakoids extended over the entire length of the chloroplast [1]. The peculiar thylakoid membrane organization in these algae raises the question of the organization of the electron-transport complexes in the thylakoid membrane. The work presented in this article provides information on the question of photosystem stoichiometry and on the chlorophyll antenna size in a unique green alga (*Dunaliella salina*). The usefulness of *D. salina* in photosynthesis research is derived from the fact that it lacks a cell wall and, therefore, it is amenable to cell fractionation without use of harsh treatments. In addition, it is capable of growth under a broad range of salt concentrations and it maintains a high glycerol content for osmotic adjustment [15].

Materials and Methods

Dunaliella salina cultures were grown in media containing 2.0 M NaCl as described [15]. Cells were inoculated into fresh media daily to ensure growth in the logarithmic phase. The cell density at the time of harvest was approx. 10^6 cells/ml. Cell cultures were maintained under incandescent illumination at 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ intensity.

Cells were harvested by centrifugation at 1500 $\times g$ for 3 min and resuspended in 50 mM Tricine (pH 7.8) containing 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, and then recentrifuged at 1500 $\times g$ for 3 min. The pellet was resuspended in a solution containing 50 mM Tricine (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (hypotonic buffer). Cells were passed twice through a Yeda press at a pressure of 13.7 MPa. This treatment, in addition to breaking the cells, resulted in shearing of thylakoid membranes (broken thylakoids). Centrifugation at 3000 $\times g$ for 5 min pelleted unbroken cells and other large fragments. The supernatant was then centrifuged at 45 000 $\times g$ for 10 min. The pellet containing the thylakoid membranes was resuspended in fresh hypotonic buffer. Chlorophyll concentration was determined in 80% acetone extract [16,17]. Immediately prior to the various measurements, the samples were diluted to 100 μM total Chl ($a + b$). Samples were always kept in the dark on ice until used.

Light-induced absorbance-difference measurements were made with a laboratory-constructed split-beam spectrophotometer. The concentration of the primary quinone electron acceptor of PS II, Q_A, was determined from the amplitude of the light-minus-dark absorbance change at 320 nm in chloroplast thylakoid membranes suspended in the presence of 20 μM DCMU and 2.0 mM potassium ferricyanide. The procedure of Pulles et al. [18] was used to correct for particle flattening effects and a differential extinction coefficient of 13 $mM^{-1} \cdot cm^{-1}$ was applied to the corrected absorbance difference at 320 nm [19]. Optical pathlength of the cuvette was 0.178 cm. Absorbance spectra of thylakoid membranes were measured according to the technique of Shibata [20], using an Aminco DW2a spectrophotometer with a 3 nm slit width and opal quartz cuvettes mounted directly against the photo-multiplier tube to minimize light scattering effects. The flattening correction factor at 320 nm was between 1.12 and 1.15 for *D. salina* thylakoids.

The concentration of the primary electron acceptor pheophytin of PS II was determined from the light-minus-dark absorbance difference change

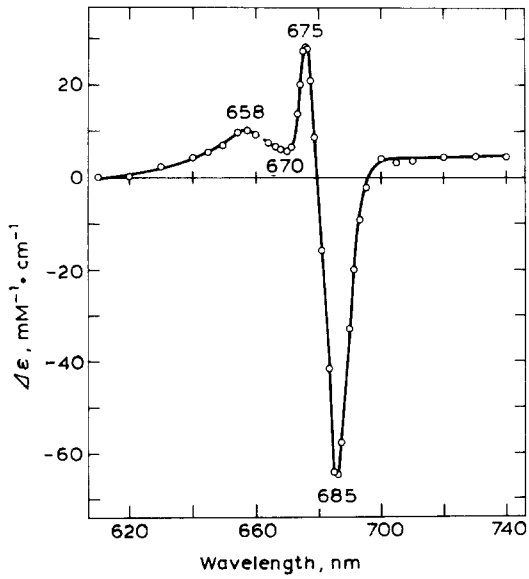

Fig. 1. Light-induced absorbance-difference spectrum derived with resolved membranes from the grana partition regions of spinach chloroplasts (BBY particles [23]). The redox potential of the suspending medium was set at −490 mV. The differential extinction coefficients were calibrated on the basis of 1 mol PS II per 230 mol Chl ($a + b$) in the preparation [22–24].

at 685 nm (ΔA_{685}) [21,22]. Light-induced absorbance change measurements of Pheo *a* were taken in an anaerobic cell of 1 cm pathlength as described [22]. In order to alleviate light scattering and sedimentation of thylakoid membranes in the cuvette, 0.005% (v/v) Triton X-100 was added to the samples prior to the measurement. The reaction mixture contained 20 mM Tris-HCl (pH 7.8), 35 mM NaCl, 2 mM $MgCl_2$, 2 μM methyl viologen, 2 μM indigodisulphonate, 2 mM $MnCl_2$ and sufficient dithionite to lower the redox potential to −490 mV. The Pheo photoreduction was induced by blue (Corning CS 4-96) excitation light at an intensity of 600 $\mu E \cdot m^{-2} \cdot s^{-1}$. The half-bandwidth of the measuring beam was 1 nm. The absorbance-difference measurements were corrected for the effect of particle flattening [18]. To determine the extinction coefficients for pheophytin photoreduction, the difference spectrum of pheophytin photoreduction was measured with resolved membranes from the grana partition regions of spinach (BBY particles [23]). Fig. 1 shows the wavelength dependence of the light-induced absorbance change obtained with BBY * particles from spinach chloroplasts suspended under reducing conditions ($E_h = -490$ mV). The difference spectrum shows minor positive bands at 658 and 675 nm and a major negative band at 685 nm. The position of the peaks and the narrow band width of the 685 nm band identify the difference spectrum as arising from the photochemical reduction of the Pheo primary electron acceptor of PS II [21]. The differential extinction coefficient of Pheo *a* in the membrane of the grana partition regions was estimated directly by using the known Chl/PS II = 230 : 1 in these membrane particles [23,24] and by assuming a 1 : 1 ratio for Pheo/PS II during photoreduction. Thus, the differential extinction coefficient of $\Delta\varepsilon_{685} = 65\ mM^{-1} \cdot cm^{-1}$ is in good agreement with the $\Delta\varepsilon$ of P-700 and of P-680 [25,26].

The concentration of the reaction center P-700 of PS I was determined from the amplitude of the light-minus-dark absorbance change at 700 nm of solubilized (0.2% SDS) chloroplast membranes suspended in the presence of 2 mM sodium ascorbate and 200 μM methyl viologen. An extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used for the calculation of P-700 concentration [25]. Optical pathlength of the cuvette was 0.178 cm.

The rate of light absorption by PS II was determined under light limiting conditions from the kinetic analysis of the area growth over the fluorescence curve of DCMU-treated (20 μM) thylakoid membranes [11]. The rate of light absorption by PS I was determined from analysis of the kinetics of the absorbance change at 700 nm, under the same excitation conditions [11]. To prevent secondary electron donation to P-700, broken thylakoid membranes were washed to remove the soluble electron donor to P-700 (removal of plastocyanin and/or soluble cytochrome *c*). This treatment resulted in the complete inhibition of all electron donation to P-700. The reaction mixture contained 25 μM DCMU and 20 μM ferricyanide or 200 μM methyl viologen. Actinic illumination of uniform field was provided by broad band green light transmitted by a combination of CS

* BBY particles are called after Berthold, Babcok and Yocum [23].

4-96 and CS 3-70 Corning filters at an intensity of 30 $\mu E \cdot m^{-2} \cdot s^{-1}$. Green light was used because it excites Chl *a* and Chl *b* about equally [27]. The functional Chl antenna size of PS II_α, PS II_β and PS I was estimated from the known ratio of total chlorophyll to a reaction center (Chl/PS I or Chl/PS II) and from the relative rates of light absorption by each photosystem. According to this approach, Chl was assigned to each photosystem in direct proportion to the measured rate. This was implemented from the solution of the following system of equations which yielded the functional antenna size (N) of PS II and PS I [12,27]:

$$\frac{\text{Chl}}{\text{PS I}} = N_\alpha \frac{[\text{PS II}_\alpha]}{[\text{PS I}]} + N_\beta \frac{[\text{PS II}_\beta]}{[\text{PS I}]} + N_I$$

$$N_\alpha = cK_\alpha$$

$$N_\beta = cK_\beta$$

$$N_I = cK_I$$

where K is the experimental rate of light absorption. The proportionality constant c depends on the quantum yield of charge separation at each photosystem. The same value of c was assumed for PS II_α, PS II_β and PS I [11,27].

Quantitation of PS II_β was obtained from the analysis of the fluorescence induction kinetics with thylakoids in the presence of DCMU [11,28]. Quantitation of PS II centers unable to reduce plastoquinone (PS II-Q_B-nonreducing) was obtained with intact *D. salina* cells in vivo from the amplitude of the initial fluorescence yield increase (F_0 to F_{pl} according to Forbush and Kok [29]) as earlier described [28].

Thylakoid membrane proteins were resolved by SDS-PAGE using the discontinuous buffer system of Laemmli [30] with a gradient resolving gel of 12.5% to 22% and a 5% stacking gel. The samples were solubilized in an equal volume of 500 mM Tris-HCl (pH 7.0) buffer containing 20% glycerol, 7% SDS, 2 M urea and 10% β-mercaptoethanol at 20 °C for 1 h. Approx. 20 μg Chl were loaded on each lane. Electrophoresis on 0.15 × 20 × 30 cm slabs was performed at a constant current of 11 mA for 18 h. The gels were stained with 0.1% Coomassie blue R.

Light harvesting complex II polypeptides were identified by Western blot analysis using a polyclonal anti-LHC II antibody raised against maize LHC II by Dr. Richard Malkin. SDS-PAGE resolved polypeptides were electrophoretically transferred to nitrocellulose and incubated with the above antibody. Bound antibody was visualized by the horse radish peroxidase-conjugated goat second antibody reaction [31]. The blots were color-developed using the manufacturer's (Bio-Rad) directions.

Results

Component quantitation

The Chl *a*/Chl *b* ratio in green algae depends on the incident light intensity during cell growth [32]. Under the experimental conditions employed in this work, the Chl *a*/Chl *b* ratio of *Dunaliella salina* was 4.4. Photosystem quantitation in *D. salina* was performed as described (Materials and Methods) from the spectrophotometric measurement of P-700, Q_A and pheophytin. Fig. 2 shows typical traces from such light minus dark measurements. Fig. 2 (upper) shows the amplitude of the light-induced absorbance decrease at 700 nm (ΔA_{700}) translating into a Chl/P-700 = 750 : 1. Fig. 2 (middle) shows the light-induced absorbance increase at 320 nm (ΔA_{320}) attributed to semiquinone anion formation, indicating a Chl/Q_A = 530 : 1. Similarly, Fig. 2 (lower) shows the absorbance decrease at 685 nm (ΔA_{685}) attributed to pheophytin photoreduction, indicating a Chl/Pheo = 540 : 1. On the basis of these measurements, the PS II/PS I stoichiometry in *D. salina* was estimated to be 1.4/1.0. This photosystem stoichiometry estimate is in good agreement with similar measurements in *Chlamydomonas* [33], in *Chlorella* [34,35], and in vascular plants grown under low-light intensity [36,37]. A summary of the quantitation data is presented in Table I.

A meaningful evaluation of photosystem stoichiometry in *D. salina* can only be reached upon addressing the question of PS II heterogeneity in this organism. Two aspects of PS II heterogeneity have been discussed in the literature. The first is a PS II antenna heterogeneity (PS II_α, PS II_β) and the second is a reducing side heterogeneity.

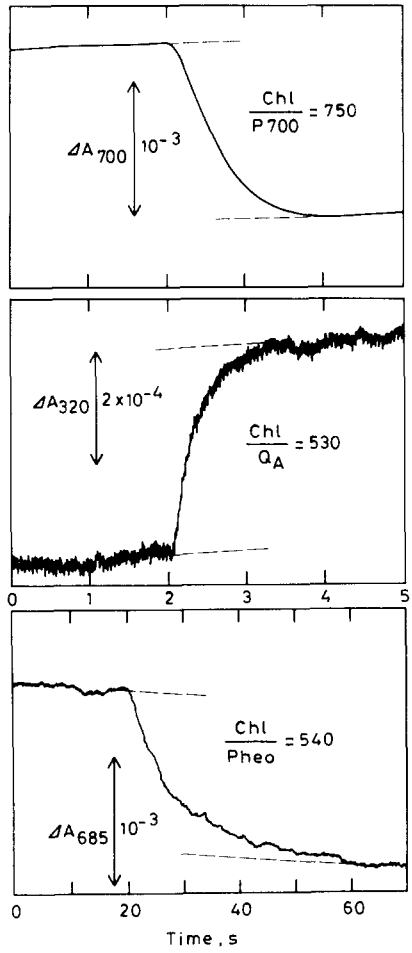

Fig. 2. Light-induced absorbance change measurements at 700, 320, and 685 nm with isolated *D. salina* thylakoids. (Upper) Determination of P-700 concentration from the amplitude of ΔA_{700}. (Middle) Determination of semiquinone anion concentration (Q_A) from ΔA_{320}. (Lower) Determination of pheophytin concentration from the amplitude of ΔA_{685}. Registration of baseline from $t = 0$ to $t = 2$ s ($t = 20$ s for ΔA_{685}). Actinic light came ON at $t = 2$ s ($t = 20$ s for ΔA_{685}). Traces represent the average of three measurements with different samples.

PS II antenna heterogeneity

The distinction between PS II_α and PS II_β was originally noted in isolated thylakoids suspended in the presence of DCMU [38]. Because of the smaller photosynthetic unit size, the activity of PS II_β was manifested as a slower exponential component in the fluorescence induction kinetics that lasted longer than the main sigmoidal component. Fig. 3 shows a typical fluorescence induction trace

TABLE I

PHOTOCHEMICAL APPARATUS CHARACTERISTICS IN *DUNALIELLA SALINA*

The relative concentration of Chl, PS I, PS II is given on a mol/mol basis. The fraction of PS II_β and PS II-Q_B-nonreducing centers is given as a percent of total PS II in the thylakoid membrane. The number of functional Chl ($a + b$) molecules associated with PS II_α, PS II_β and PS I is given by N_α, N_β and N_I, respectively.

Chl *a*/Chl *b*	4.4
Chl/PS I	750:1
Chl/PS II	535:1
PS II/PS I	1.4:1
PS II_β	45%
PS II-Q_B-nonreducing	25%
N_α	570
N_β	150
N_I	220

obtained with *D. salina* thylakoids suspended in the presence of 20 μM DCMU. The shaded area reflects the exponential phase of the fluorescence induction trace where the activity of PS II_β is not hindered by the fast component. This was confirmed in the semilogarithmic plot of the area over fluorescence induction kinetics (Fig. 3, inset). The

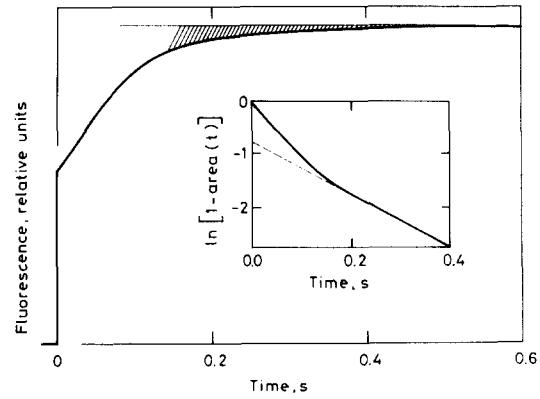

Fig. 3. Fluorescence induction kinetics with isolated *D. salina* thylakoids suspended in the presence of 20 μM DCMU. The shaded area marks the portion of the fluorescence induction trace where the activity of PS II_β is not hindered by the fast induction component. Inset: Semilogarithmic analysis of the area growth over fluorescence induction kinetics revealing the biphasic nature of the phenomenon. The slow linear phase (PS II_β) accounted for about 45% of total area growth. Deconvolution of the kinetics defined rates of light absorption by PS II_α ($K_\alpha = 18.4\ s^{-1}$) and PS II_β ($K_\beta = 4.9\ s^{-1}$).

biphasic kinetics defined a slow component (PS II_β) accounting for about 45% of the area growth. This estimate of the concentration of PS II_β in *D. salina* (45%, based on antenna size) is consistent with similar measurements in *Chlamydomonas* [33].

PS II reducing side heterogeneity

Evidence has been presented in the literature suggesting that a number of PS II reaction centers in chloroplasts, although photochemically competent, are unable to transfer electrons efficiently from Q_A^- to Q_B [28,39–41]. Following the nomenclature of Lavergne [40], these centers are termed PS II-Q_B-nonreducing in order to distinguish them from the PQ-reducing PS II centers (Q_B-reducing). It is possible to monitor both the activity and relative concentration of PS II-Q_B-nonreducing centers in vivo, i.e., in intact leaf discs or algal cells in the absence of herbicides [28]. The method is based on the inability of these centers to transfer electrons from Q_A^- to Q_B, hence, upon illumination their photochemical activity is revealed by a fluorescence yield increase from F_0 to the intermediate plateau F_{pl} [29]. An example of this experimental approach is provided in Fig. 4, where the fluorescence induction trace of *D. salina* thylakoids is presented. The initial fluorescence yield increase from F_0 to F_{pl} precedes the main variable fluorescence rise to F_{max} [42,43]. The relative fluorescence yield of the transition from F_0 to F_{pl} provides a measure of the relative concentration of PS II-Q_B-nonreducing. Fig. 5 compares fluorescence induction traces obtained with intact cells of *D. salina* suspended in the presence and absence of DCMU. Fig. 5 (+DCMU) shows the sigmoidal fluorescence yield increase attributed to the photoreduction of Q_A in all PS II centers. Fig. 5 (−DCMU) shows the initial portion of the fluroescence induction (F_0 to F_{pl}) which is an exponential function of time, attributed to the photoactivity of PS II-Q_B-nonreducing [28]. The amplitude of the F_0 to F_{pl} fluorescence yield increase (−DCMU) is approximately equal to 25% of the total variable fluorescence yield measured in the presence of DCMU, indicating that about 25% of all photosystem II centers in *D. salina* are unable to reduce the plastoquinone pool (PS II-Q_B-nonreducing).

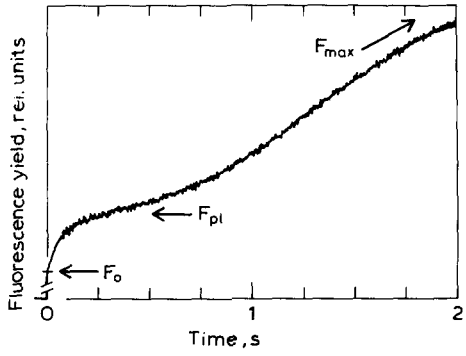

Fig. 4. Fluorescence induction kinetics with isolated *D. salina* thylakoids suspended in the absence of PS II inhibitors.

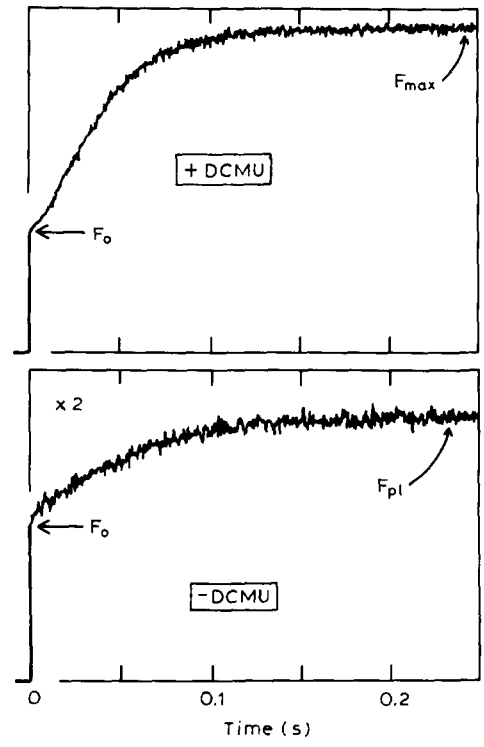

Fig. 5. Fluorescence induction kinetics with intact *D. salina* cells suspended either in the presence (upper) or absence (lower) of DCMU. The actinic light came on at zero time. The F_0 to F_{pl} fluorescence yield increase (−DCMU) preceded the main variable fluorescence rise to F_{max} (not shown). The apparatus gain was set 2× for the −DCMU trace compared to 1× for the +DCMU trace.

Functional antenna size

Elucidation of the photochemical apparatus organization requires knowledge of the photosys-

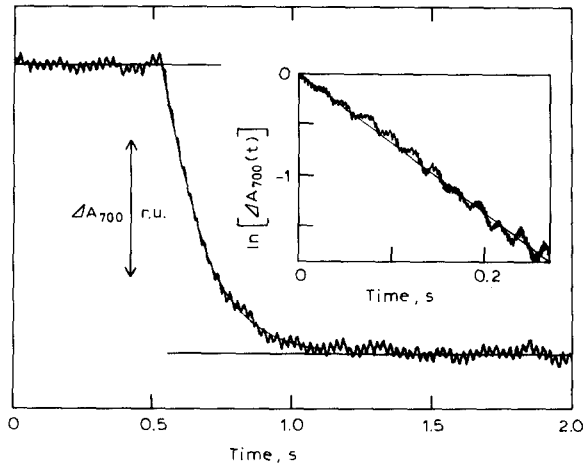

Fig. 6. Absorbance change kinetics of P-700 (ΔA_{700}) upon illumination of isolated *D. salina* thylakoids. Broken thylakoid membranes were washed to remove the soluble donor to P-700 (plastocyanin or cytochrome *c*), thus preventing secondary electron donation to P-700$^+$. The actinic light came on at 0.55 s. Inset: Semilogarithmic plot of the absorbance change kinetics. The slope of the straight line defined the rate of light absorption by PS I ($K_I = 7.2\ s^{-1}$). The trace represents the average of four measurements with two separate samples.

tem stoichiometry in the thylakoid membrane and knowledge of the chlorophyll antenna size and composition of each photosystem. The question of photosystem stoichiometry in *D. salina* was addressed in the foregoing. The question of the chlorophyll antenna size of each photosystem can be addressed from the relative rates of light absorption by each photosystem and from the total Chl ($a + b$) per PS I and per PS II in the thylakoid membrane (Table I). It is reasoned that, under limiting intensity conditions, the rate of light absorption by each photosystem would be directly proportional to its Chl antenna size (see Materials and Methods). Typical kinetic traces of PS II and PS I photoactivity are shown in Fig. 3 and Fig. 6, respectively. Fig. 3 shows a typical fluorescence induction trace and the semilogarithmic plot of the area over the fluorescence induction (inset). Analysis of the biphasic PS II kinetics (not shown) yielded the rates of light absorption by PS II_α and PS II_β ($K_\alpha = 18.4\ s^{-1}$ and $K_\beta = 4.9\ s^{-1}$).

The rate of light absorption by PS I was determined from the kinetics of P-700 photooxidation in isolated thylakoid membranes of *D. salina* in which secondary electron donation to P-700 was blocked. Fig. 6 shows the kinetics of P-700 photooxidation and a semilogarithmic plot of the kinetic data (inset). The slope of the semilogarithmic straight line defined an apparent rate of light absorption by PS I ($K_I = 7.2\ s^{-1}$).

Based on the above rate constants and the Chl/PS I and Chl/PS II ratios shown in Table I, the functional antenna size of PS II_α, PS II_β and PS I was estimated as 570, 150 and 220 Chl ($a + b$) molecules, respectively. The PS II_β and PS I antenna sizes of *D. salina* are comparable to those of higher plant chloroplasts [11]. However, the PS II_α antenna size in *D. salina* is considerably larger than that of higher plants.

Polypeptide composition

The polypeptide composition of the thylakoid membrane in *D. salina* was compared to that of

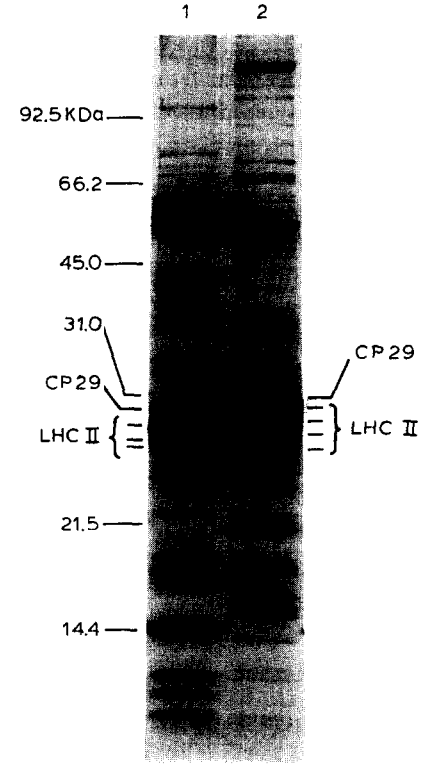

Fig. 7. Electrophoretic pattern of Coomassie stained polypeptides in SDS-PAGE analysis of isolated thylakoid membranes from spinach (lane 1) and from *D. salina* (lane 2). Lanes were loaded with 20 μg Chl ($a + b$). Polypeptides in the 25–29 kDa region are associated with the Chl *ab* LHC II, evidenced by Western blot analysis (not shown). The LHC II appears to contain at least three distinct polypeptides in spinach (lane 1) and four distinct polypeptides in *D. salina* (lane 2).

spinach chloroplasts by SDS-PAGE analysis. Fig. 7 shows the total thylakoid membrane protein profile of *D. salina* and spinach. An area of interest is the 25–35 kDa region where polypeptides of the LHC II migrate. One distinct difference between the two samples is the slower electrophoretic mobility of LHC II polypeptides in *D. salina*. Another difference is the number of polypeptides associated with the LHC II antenna. In addition to CP29, spinach chloroplasts have three polypeptides associated with LHC II, whereas *D. salina* appears to have four polypeptides associated with the LHC II antenna. Densitometric scans of the 25–35 kDa region in Coomassie-stained gels revealed that, when loaded on an equal Chl basis, the integrated area of LHC II polypeptides in *D. salina* was on the average greater by about 22% than that of spinach.

Discussion

The PS II/PS I stoichiometry in the thylakoid membrane of *D. salina* was 1.4/1.0, suggesting that green algae, much like higher plant chloroplasts, have non-stoichiometric amounts of photosystem complexes in the thylakoid membrane. However, the measurement of PS II/PS I = 1.4/1.0 in *D. salina* must be further qualified (a) because of the dependence of the PS II/PS I ratio on the light intensity during cell growth, and (b) in view of the phenomenon of PS II heterogeneity.

Work from several laboratories suggested a dependence of the PS II/PS I ratio on the light intensity during plant growth. The cause-and-effect relationship between light intensity and photosystem stoichiometry is not currently understood. It appears that both higher plants [36,37] and green algae [33,35] develop a higher PS II/PS I ratio under high light intensity conditions. Hence, a PS II/PS I = 1.4/1.0 in *D. salina* should not be considered as a fixed value but one that reflects the light regime during cell growth (an apparent exception to this notion was observed in *Dunaliella tertiolecta* [44]).

Analysis on PS II heterogeneity presented in this work (Fig. 3) suggested that approx. 45% of all PS II centers were PS II_β. The latter are distinguished from PS II_α by the smaller photosynthetic unit size; PS II_β appears to lack the LHC II-peripheral antenna. Further analysis (Fig. 5) revealed that approx. 25% of all PS II centers in *D. salina* were unable to transfer electrons efficiently from Q_A^- to Q_B (PS II-Q_B-nonreducing). The existence of a significant pool of PS II centers that are photochemically competent but unable to transfer electrons to PQ probably has an overall adverse effect on PS II electron transport; it would lower the operational PS II/PS I ratio from 1.4/1.0 to about 1.05/1.0. There is an interrelationship between PS II_β and PS II-Q_B-nonreducing. Both appear to display exponential fluorescence induction kinetics, suggesting that they are isolated units (Figs. 3 and 5). Earlier work with spinach indicated (a) exponential fluorescence induction kinetics for both PS II_β and PS II-Q_B-nonreducing; (b) the fluorescence yield amplitude controlled by PS II_β (F_{v_β}) was identical to that controlled by PS II-Q_B-nonreducing (F_0 to F_{pl}); and (c) the fraction of Q_A corresponding to PS II_β was equal to the amount of Q_A photoreduced during the F_0 to F_{pl} transition. On the basis of these findings, it was suggested that in mature spinach chloroplasts PS II_β and PS II-Q_B-nonreducing constitute one and the same pool of PS II centers [28]. In *D. salina* there is a significant overlap between PS II-Q_B-nonreducing centers (25% of the total PS II) and PS II_β (approx. 45% of the total PS II), suggesting that PS II-Q_B-nonreducing is a subpopulation of PS II_β in the thylakoid membrane of this alga. The greater fraction of PS II_β than PS II-Q_B-nonreducing in *D. salina* may indicate the presence of PS II lacking LHC II-peripheral but active in the process of PQ reduction. One may argue this is an artifact during thylakoid membrane isolation resulting in the energetic uncoupling of the LHC II-peripheral from PS II_α units. Alternatively, it may be argued that *D. salina* cultures with cells in the logarithmic phase of growth, have chloroplasts in the developing stage. It is well known that chloroplasts in the developing stage have not yet accumulated their full complement of LHC II-peripheral antenna and, therefore, they display enhanced concentrations of PS II_β which is active in the process of PQ reduction [12–14]. This contention is supported by the work of Krupinska and Senger [45] who employed synchronously grown cultures of *Scenedesmus* and found variations in the PS II_α/PS II_β

ratio depending on the stage of the cell cycle. Hence, the generalization that PS II_β under all circumstances has an impaired Q_A–Q_B interaction should not be made. Notable exceptions have been reported in Chl *b*-deficient mutants in which PS II_β (antenna size of about 130 Chl $(a+b)$ molecules) constitutes up to 80% of all PS II, most of which is active in the process of electron transport to plastoquinone [12–14]. Concerning the physiological significance of PS II heterogeneity in photosynthetic tissues, it is possible that PS II-Q_B-nonreducing represents a state of repair of the PS II unit in which the reaction center has replaced the damaged 32 kDa Q_B-binding protein. This hypothesis is consistent with the observation that PS II-Q_B-nonreducing centers are localized in stroma-exposed regions of the thylakoid lamella, they lack the LHC II-peripheral, and have not yet established a functional interaction between Q_A^- and Q_B [28]. It is also consistent with the rapid turnover of the 32 kDa (PS II reaction center, or D1) protein and with the evidence that newly synthesized 32 kDa polypeptides first 'assemble' in stroma thylakoids and subsequently move into the grana [46]. Conversely, PS II_β is defined solely by the smaller Chl antenna size (lack of LHC II-peripheral), without reference to electron-transport properties on the reducing side of the reaction center. A summary of the two aspects of PS II heterogeneity, as discussed in this work, appears in Fig. 8.

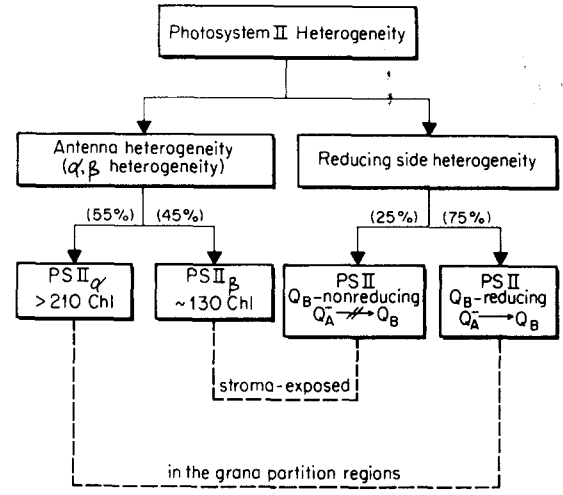

Fig. 8. Schematic delineating the two aspects of Photosystem II heterogeneity discussed in the literature. In wild-type mature chloroplasts from higher plants and green algae there is a PS II antenna heterogeneity (α,β-heterogeneity). This reflects the presence of a dominant PS II_α, which is localized in the membrane of the grana partition region and contains 210 or more Chl $(a+b)$ molecules per reaction center. Photosystem II_β is localized in stroma-exposed lamellae and lacks the LHC II-peripheral, resulting in a smaller antenna size of about 130 ± 20 Chl $(a+b)$ molecules. A reducing side heterogeneity reflects the presence of a small pool of PS II centers impaired in the Q_A-Q_B electron-transport process (Q_B-nonreducing). In wild type mature chloroplasts, PS II_β and PS II-Q_B-nonreducing may constitute one and the same pool of PS II centers [28]. However, this strict relationship does not hold in developing chloroplasts. In *D. salina,* PS II-Q_B-nonreducing is a subpopulation of PS II_β.

Estimation of the Chl antenna size of PS I and PS II was based on the measurement of the total Chl per PS I and per PS II centers (Chl/P-700, Chl/Pheo, Chl/Q_A shown in Fig. 2), and on the apportionment of the total Chl to PS I and PS II centers. The distribution of the total Chl into PS I and PS II centers was done in direct proportion to the photoactivity of each reaction center. The latter was measured kinetically from the P-700 photooxidation and Q_A photoreduction (Figs. 3 and 6). In the past, this method predicted a PS II_α antenna of 230 Chl $(a+b)$ and a PS I antenna of 210 Chl $(a+b)$ molecules in spinach [11,12]. This was subsequently verified upon the isolation of resolved membranes from the grana partition regions (BBY particles [24]) and of a 'native' PS I complex [5,47]. Moreover, a PS I antenna size of 140 Chl *a* molecules was measured in *Synechococcus* 6301 thylakoids [10], and was later confirmed in isolated PS I complexes from cyanobacterial thylakoids [48,49]. The results from this work support the notion of a PS I and PS II_β antenna size in *D. salina* ($N_I = 220$ and $N_\beta = 150$ Chl molecules) comparable to those of higher plant chloroplasts. However, the antenna size of PS II_α ($N_a = 570$) in *D. salina* appeared considerably greater than that of higher plant chloroplasts. This is consistent with the result of similar measurements in *Chlamydomonas* [33] and suggests differences in the organization of the LHC II between *D. salina* and higher plant chloroplasts. Indeed, based on the antenna size measurements given above and on the apparent Chl *a*/Chl *b* = 4.4 in *D. salina,* we estimated a molecular ratio of Chl *a*/Chl *b* between 2.5 and 3.0 for the purified Chl *ab* LHC

II. This estimate is in sharp contrast with the nearly equimolar amounts of Chl *a* and Chl *b* (Chl *a*/Chl *b* = 1.17) found in the LHC II of higher plants, but consistent with the results of Sukenik et al. in *D. tertiolecta* [44] and of Pick et al. in *D. salina* [50].

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