Biochimica et Biophysica Acta, 1100 (1992) 83–91

BBABIO 43591

Restoration of irradiance-stressed *Dunaliella salina* (green alga) to physiological growth conditions: changes in antenna size and composition of Photosystem II

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(Received 28 September 1991)

Key words: Adaptation mechanism; Chlorophyll-protein complex; Fluorescence; Light-ha PAGE, non-denaturing; Photosystem heterogeneity; Photosystem II; (*D. salina*)

The green alga *Dunaliella salina* responds to growth under high-intensity light by reducing the complement of auxiliary light-harvesting complexes integrated into the thylakoid membrane, giving a reduction in the functional chlorophyll antenna size for both photosystems. Acclimation of the antenna system of Photosystem II upon removal of high-light-adapted cells to a low-light regime was found to occur as a distinct sequence of reassembly events. Analysis of fluorescence-induction kinetics indicated a conversion step after 4 h of low-light acclimation in which PS II_γ centres were converted to PS II_β centres. Conversion of PS II_β centres to PS II_α centres proceeded after 12 h and was accompanied by an increase in PS II-variable fluorescence yield and PS II-quantum yield. Changes in chlorophyll *a* binding by the CP47 polypeptide of the PS II core preceded changes in membrane protein composition and reassembly of auxiliary antenna complexes, suggesting that regulation of assembly of the PS II antenna occurs at the level of chlorophyll synthesis. Chlorophyll binding by CP47 is not essential for its primary function in assembly of the complete photosynthetic unit. A model for the PS II antenna in which CP47 and CP43 act independently for excitation energy transfer to the PS II reaction centre is proposed and its implications are discussed.

Introduction

Photosystem I and Photosystem II are integral thylakoid membrane complexes each containing a reaction centre complex, which binds chlorophyll (Chl) *a* and the other electron transport intermediates involved in primary photochemical events, and a light-harvesting pigment-protein antenna complex which functions to absorb light energy and transfer the excitation to the reaction centre (reviewed in Ref. 1). The light-harvesting complexes of PS I and PS II are termed LHC-I and LHC-II, respectively, and these are exclusively responsible for binding the accessory pigment Chl *b* within the thylakoid membrane. Changes in thylakoid membrane Chl *a*/*b* ratio, which occur naturally during chloroplast development and in response to environmental conditions [2], are therefore indicative of changes in the complement of accessory light-harvesting proteins within the thylakoid membrane [3–5].

Photosynthetic organisms are able to respond to variations in both the spectral quality and the intensity of light under which they are grown by adjusting the composition and structure of the photosynthetic apparatus [2]. Such adjustments include alterations in photosystem stoichiometry as well as in the complement of accessory light-harvesting proteins associated with each of the two types of photosystem. In general, growth under low intensity illumination induces an increase in the number of light-harvesting complexes associated with each photosystem, whereas growth under high irradiance causes a depletion in the number of light-harvesting complexes serving each photosystem. Such

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Abbreviations: A_m, unnormalized area above the fluorescence induction curve; A_t, area above the fluorescence induction curve after time *t*; Chl, chlorophyll; CP, chlorophyll-protein; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea, F_m, maximal fluorescence occurring when all PS II reaction centres are closed; F_0, minimal fluorescence occurring when all PS II centres are open; F_v, variable fluorescence $F_v = F_m - F_0$; LHC-I, the Chl *a*/*b*-binding light-harvesting complex of PS I; LHC-II, the Chl *a*/*b*-binding light-harvesting complex of PS II; PAGE, polyacrylamide gel electrophoresis; PS I, Photosystem I; PS II, Photosystem II; S(L)DS, sodium (lithium) dodecyl sulphate.

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variations are reflected as changes in the functional antenna size, a measure of the total number of chlorophyll molecules conducting excitation energy to each photosystem reaction centre. Assembly of the photosynthetic apparatus under conditions of variability in incident illumination does not appear to be regulated by a mechanism involving the phytochrome response [2,6,7], and may instead be regulated at the level of chlorophyll biosynthesis [8].

The halotolerant unicellular green alga *Dunaliella salina* shows a remarkable capacity for acclimation to diverse intensities of incident illumination [9,10]. Under normal growth conditions, the organism exhibits a Chl *a*/*b* ratio of about 4.0. The population of PS II centres within the thylakoid membrane is heterogeneous with respect to photosynthetic unit size [11,12], with the predominant form of centre, PS II_α, having an antenna size which may be some 260 chlorophyll molecules, approximately twice that of the minority PS II_β form [10,13,14]. Under conditions of high irradiance, the organism exhibits a high Chl *a*/*b* ratio (≥ 20), reflecting a greatly reduced complement of accessory light-harvesting proteins associated with each photosystem. In the thylakoid membranes of *D. salina* cells grown under high-light (HL) conditions, a population of PS II centres with an antenna size of only 60 chlorophyll molecules predominates. These centres, termed PS II_γ [10], therefore, have a residual antenna size approximately half that of PS II_β centres.

Despite advances in our understanding of the antenna composition of the photosynthetic apparatus (see ref. 15 for a recent review), questions still arise concerning assembly of PS II light-harvesting complexes and their organisation and orientation relative to each other and to the PS II reaction centre. Questions also arise surrounding the structural differences in the antennae of the PS II subpopulations. In this study, we have exploited the plasticity which occurs in the organisation of the photosynthetic apparatus in *D. salina* to investigate the assembly and molecular architecture of the antenna system of PS II. Distinct functional changes in the antenna of PS II were observed to occur in discrete stages during the restoration to low-intensity light of *D. salina* cells previously acclimated to high-intensity light. These observed changes reflect progressive steps in the reassembly of the PS II antenna system, and their implications for a general model describing the structure of the PS II antenna are discussed.

Materials and Methods

Cell culture

D. salina cells were grown at 28°C in a medium containing 2.0 M NaCl with CO_2 supplied as 20 mM sodium hydrogen carbonate [10]. For low-light (LL) growth, cultures were maintained under incandescent lights giving a photon flux density of 100 $\mu E\ m^{-2}\ s^{-1}$. For HL growth, cultures were maintained under a photon flux density of 2150 $\mu E\ m^{-2}\ s^{-1}$, provided by a tungsten-halogen discharge tube separated from the cultures by a transparent water-circulating cooling device. HL cultures were maintained at a Chl *a* concentration of not more than 1 $\mu g\ ml^{-1}$ in order to prevent self-shading or light gradient effects. For experiments in which HL cells were restored to LL, HL cells from a single stock culture were transplanted into identical subcultures at equal initial Chl *a* concentrations. These cultures were maintained under the HL regime for a further 48 h prior to removal to the LL regime. HL-grown cells acclimated to LL for 44 h were identical to LL-grown cells in all characteristics investigated.

Thylakoid membrane isolation

Thylakoid membranes were isolated from cells harvested by centrifugation at $6500 \times g$ for 10 min. Cells were resuspended in buffer containing 100 mM sorbitol, 10 mM $MgCl_2$, 5 mM NaCl, 50 mM Tricine-NaOH (pH 7.8) and were disrupted by extrusion through a French pressure cell at 250 lb/in^2. Unbroken cells and cell debris were removed by centrifugation at $6500 \times g$ for 15 min. Thylakoid membranes were harvested by centrifugation at $30000 \times g$ for 30 min, resuspended in the above buffer and maintained on ice in the dark until required.

Polyacrylamide gel electrophoresis

For non-denaturing PAGE, isolated thylakoid membranes were resuspended to a chlorophyll concentration of 1 mg ml^{-1} in 10% (v/v) glycerol, 62.5 mM Tris-HCl (pH 6.8) prior to the addition of dodecyl maltoside to a protein : detergent ratio of 5 : 1. Samples were maintained on ice in darkness with frequent agitation for 20 min. Insoluble material was removed by centrifugation at $24000 \times g$ for 10 min. Non-denaturing PAGE was performed on 8.5%–12.5% acrylamide gradient gels (8 cm × 6 cm × 0.75 mm) essentially as in Ref. 16, using an LKB minigel system cooled to 4°C by a refrigerated water-circulating system. Cathodic buffer contained 0.1% (w/v) LDS. Electrophoresis was performed at 6 mA constant current per gel for 20 min followed by 12 mA constant current per gel for a further 70 min. Two identical gels could be run simultaneously.

Molecular masses of chlorophyll-protein complexes isolated by non-denaturing PAGE were estimated by comparison of their relative electrophoretic migrations with those of standard proteins. Gel lanes containing standard proteins were rapidly stained and destained and the lane realigned with the unstained gel containing chlorophyll-proteins.

SDS-PAGE was performed on 12.5–25% acrylamide gradient gels using the buffer system of Ref. 17. Thylakoid membrane samples were solubilized at room temperature in buffer containing 10% (v/v) glycerol, 4% (w/v) SDS, 1% (w/v) β-mercaptoethanol, 62.5 mM Tris-HCl (pH 6.8). Gels were stained with Coomassie brilliant blue.

Chlorophyll concentration was determined as in Ref. 18. Protein concentration was determined for SDS-solubilized proteins by the fluorescamine-binding method described in Ref. 19.

Electrophoretic transfer of polypeptides onto nitrocellulose and subsequent immunoblotting with polyclonal antibody raised against spinach LHC-II were performed using standard procedures. Colour development with alkaline phosphatase-conjugated secondary antibody was performed as described in Ref. 20.

Low temperature fluorescence spectroscopy

Spectra were recorded at 77 K with a Perkin-Elmer LS5 luminescence spectrometer. Whole cells of *D. salina* were resuspended in fresh growth medium to a chlorophyll concentration of 5 μg ml^{-1}, dark-adapted for 15 min and rapidly frozen in liquid nitrogen. Spectra were normalized to the fluorescence emission maximum in each case.

Room temperature fluorescence induction

Room temperature PS II fluorescence induction was measured in whole cells harvested by centrifugation at 6500 × *g* for 10 min and resuspended in fresh culture medium to a chlorophyll concentration of 10 μg ml^{-1}. Actinic light was provided via fibre-optic cable from a 250 W stabilized light source, with wavelength defined by Corning 4-96 filter, and was admitted to the measuring cuvette by electronic shutter. Fluorescence signal was detected by a photodiode screened by a Schott RG665 filter, and the amplified signal was stored using a storage oscilloscope, and induction transients were plotted using an *X–Y* plotter. Cells were stirred in the dark for 10 min prior to the addition of DCMU (20 μM), which took place 5 s before switching off the stirrer and recording the transient. Kinetics of fluorescence rise were analysed as in Ref. 11.

Results

Growth of *D. salina* under high intensity light results in a cell type which exhibits a high Chl *a*/*b* ratio, as illustrated in Fig. 1a which shows changes in cellular chlorophyll content occurring when cultures were removed to a LL regime. Chl *a* concentration within cultures increased 2-fold during the 48-h period prior to removal to LL, indicating that HL exposure does not inhibit cell growth (data not shown). HL cells showed a Chl *a*/*b* ratio of 25. Removal to a LL regime induces an immediate increase in the total cellular chlorophyll (Fig. 1a, open circles), although for approximately 4 h this increase was not accompanied by any decrease in the chlorophyll *a*/*b* ratio (Fig. 1a, closed circles), suggesting a lag period preceding an accelerated synthesis of Chl *b* and its incorporation into LHCs. A 4-h lag period also preceded major changes in the protein/chlorophyll ratio of thylakoid membranes isolated from HL-cells acclimating to LL, as shown in Fig. 1b, supporting the suggestion that assembly of new LHCs into the thylakoid membrane does not occur for at least some 4 h after removal to LL.

Changes occurring in the organisation of the thylakoid membrane of HL-grown *D. salina* cells acclimating to LL were investigated by fluorescence spectroscopy at 77 K. Fluorescence emission spectra derived from cells acclimated to LL for varying times are shown in Fig. 2. HL-grown cells show distinct emission maxima at 683 nm and 710 nm. These emission maxima are attributable to the antenna-depleted PS II core [21] and PS I core [22], respectively.

The most striking feature of the spectrum derived from HL-grown cells acclimated to LL for 4 h is the appearance of a prominent emission peak at 695 nm (Fig. 2). The ratio of 710 nm emission to 683 nm emission is, however, unchanged after 4 h. Subsequent to this, fluorescence emission at 683 nm becomes predominant, presumably as a result of the assembly of LHC-II into the thylakoid membrane, which is implied

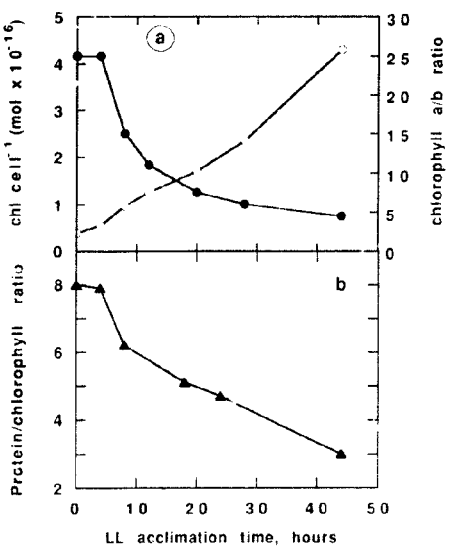

Fig. 1. (a) Changes in cellular chlorophyll (○) and Chl *a*/*b* ratio (●) during acclimation to LL of HL-grown *D. salina*. (b) Thylakoid membrane protein/chlorophyll ratio during LL acclimation of HL-grown *D. salina* cells. HL-grown cells were removed to LL at time zero.

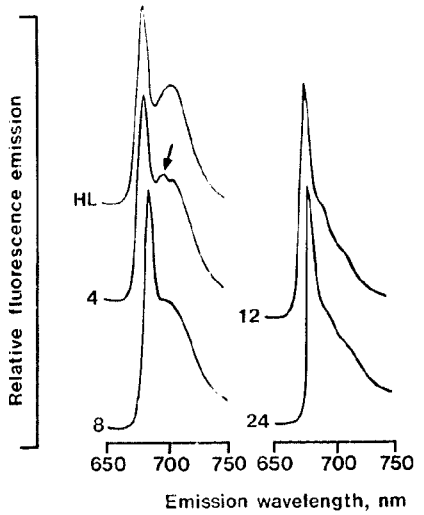

Fig. 2. Fluorescence emission spectra recorded at 77 K for HL-grown cells of *D. salina* acclimated to LL for varying times indicated. Emission in the wavelength range 650–750 nm was detected with excitation through Chl *a* at 435 nm. Spectra were normalised to the emission maximum at 683 nm in each case, but are drawn apart for clarity. The distinct fluorescence component at 695 nm and arising during the initial 4 h of LL acclimation is indicated by the arrow.

by the observed decrease in Chl *a*/*b* ratio occurring over the time course of LL acclimation.

Fluorescence emission at 695 nm has been assigned to the CP47 Chl *a*-binding polypeptide which is a component of the PS II core [23,24]. The increased emission at 695 nm which occurs at this early stage in acclimation to LL could arise from increased abundance of the CP47 chlorophyll-protein complex emitting at this wavelength, or increased chlorophyll-binding to the complex very early after removal to LL. Since changes in Chl *a*/*b* ratio are not observed at this early stage in acclimation to LL, it is unlikely that increased 695 nm emission arises directly from increased abundance of a Chl *a*/*b*-binding species or indirectly as a result of increased excitation energy transfer from such a species to the complex emitting at 695 nm. It is, however, possible to discriminate between these possibilities by the use of fluorescence excitation spectroscopy.

Fig. 3a shows excitation spectra in the wavelength range 400–500 nm for emission at 695 nm. Prominent excitation maxima at 435 nm and 476 nm are evident, arising as a result of the absorbance by Chl *a* and Chl *b*, respectively. The ratio of excitation at 435 nm to that at 476 nm therefore provides an index of the relative contributions of excitation absorbed through Chl *a* or Chl *b* to fluorescence emission at any given wavelength. If increased emission at 695 nm at an early stage in LL acclimation results either directly or indirectly from increased levels of a Chl *a*/*b*-binding complex, it can be predicted that the relative contribution of Chl *b*-absorbed light (at 476 nm) to the emission at 695 nm would remain constant or increase during acclimation to LL. Only if the increased 695 nm emission after 4 h LL acclimation were due to an increase in levels of a pigment-protein which binds exclusively Chl *a* could it be predicted that the relative contribution of Chl *b*-absorbed excitation (at 476 nm) would decrease during the early stages of LL acclimation. This would be indicated by a transient decrease in the amplitude of the 476 nm excitation peak. From the spectra in Fig. 3a, it is clear that the relative contribution of Chl *b*-absorbed light to fluorescence emission at 695 nm does decrease during the first 4 h of LL acclimation. Changes in the ratio Ex 435 nm/Ex 476 nm, indicative of changes in the relative contributions of Chl *a* and Chl *b* to fluorescence emission at both

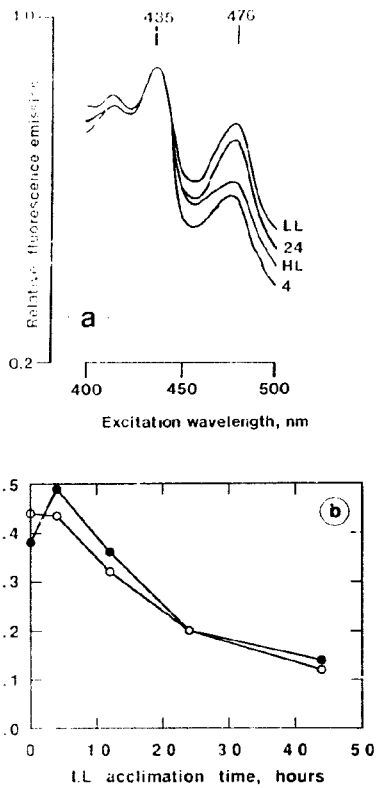

Fig. 3. (a) Excitation spectra for fluorescence emission at 695 nm, recorded at 77 K in the wavelength range 400–500 nm, from HL-grown *D. salina* acclimating to LL for varying times indicated. Spectra were normalised to the excitation maximum at 435 nm. Spectra from cells acclimated to LL between 4 and 24 h are omitted for clarity. (b) Changes in the relative contributions of excitation absorbed through Chl *a* (at 435 nm) and through Chl *b* (at 476 nm) to the fluorescence emission components at 695 nm (●) and 683 nm (○), expressed as the ratio Ex 435/Ex 476.

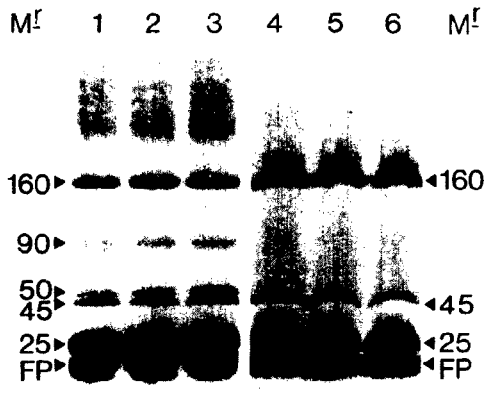

Fig. 4. Non-denaturing PAGE of dodecyl maltoside-solubilised chlorophyll-protein complexes from the thylakoid membranes of *D. salina* grown under HL (lanes 4–6) or grown under HL and reacclimated to LL for 12 h (lanes 1–3). Lanes were loaded with thylakoid material equivalent to 3 μg Chl (lanes 1, 6), 4 μg Chl (lanes 2, 5) or 5 μg Chl (lanes 3, 4). Estimated molecular masses of the chlorophyll-protein complexes are shown (M). FP indicates free pigment.

695 nm and 683 nm, are shown in Fig. 3b. For emission at 695 nm (Fig. 3b, closed circles), an initial increase in the ratio Ex 435 nm/Ex 476 nm is observed in the first 4 h of acclimation, indicating a transient increase in the relative contribution of excitation through Chl *a*. This is followed by a decrease in the ratio, indicating a subsequent progressive increase in the relative contribution of Chl *b*-absorbed excitation. For emission at 683 nm, there is no change in the ratio Ex 435 nm/Ex 476 nm over the initial 4 h of reacclimation (Fig. 3b, open circles). Subsequently, the same progressive, non-incremental decrease in the ratio occurred, as observed for emission at 695 nm. This change can therefore be attributed to an increased contribution to the 683 nm emission by excitation through LHC-II.

The data in Fig. 1 would tend to suggest that significant changes in the protein composition of the thylakoid membrane do not occur during the initial 4 h of LL acclimation in HL-grown *D. salina* cells. However, changes in distribution of chlorophyll among thylakoid membrane complexes could occur. Fig. 4 shows non-denaturing PAGE of dodecyl maltoside-solubilized chlorophyll-protein complexes from the thylakoid membranes of HL-grown *D. salina* cells and from cells acclimated to LL for 12 h. Thylakoid membranes were solubilized to the same detergent:protein ratio (see Fig. 1b), rather than the more usual index of detergent:chlorophyll ratio, in order to eliminate as much as possible any variability in resolved chlorophyll-protein complexes resulting from differential solubilization effects. Six types of chlorophyll-protein complex were resolved from the thylakoid membrane of LL cells. Complexes with approximate molecular masses 250–500 kDa, 160 kDa and 90 kDa were identified as multimeric forms of PS I, the dimeric form of the PS I reaction centre and a multimeric form of LHC-II, respectively, by 77 K fluorescence spectroscopy and polypeptide analysis by denaturing SDS-PAGE of the isolated complexes (data not shown). A broad band containing complexes of approximate mass 25 kDa contained monomeric LHCs. Two bands of mass 50 kDa and 45 kDa contained Chl *a*, corresponding to the PS II core proteins CP47 and CP43, respectively [16,25].

The multimeric forms of PS I and LHC-II were not resolved from the thylakoids of HL-grown cells, as might be expected in the light of the observed reduction in photosynthetic unit size for both photosystems under these growth conditions [10]. The most striking feature, however, was the apparent absence of chlorophyll *a* associated with a CP47 band in HL-grown cells. Since changes in protein levels are not implicated, this observation strongly suggests that changes in 77 K fluorescence emission at 695 nm occurring within four hours of commencement of LL acclimation of HL-grown cells occur as a result of increased assembly of chlorophyll *a* into CP47 polypeptides.

Changes in PS II fluorescence yield in the presence of DCMU at room temperature are also diagnostic for variations in functional properties of PS II. Fig. 5a shows plots of PS II fluorescence yield induced from HL-grown cells and HL-grown cells acclimated to LL for varying times. Fig. 5b shows the first-order kinetic analyses of the rate of growth of area above the fluorescence induction curves shown in Fig. 5a. Derived fluorescence parameters F_m/F_0 and F_v/F_m are shown in Fig. 5c, in addition to the percentage of PS II centres which are β-centres (Fig. 5c, triangles), this value being derived from the intercept at time zero of the nearly extrapolated exponential phase of the kinetic plots in Fig. 5b. HL-grown cells exhibit a low-variable fluorescence yield, indicative of the reduced antenna size of PS II under the HL conditions. Growth under HL has been found to induce a chronically photoinhibited state [10], and in this study, HL cells also showed the low F_v/F_m ratio indicative of the low quantum yield of PS II photochemistry which has been attributed to the existence of a population of photoinhibited PS II centres under HL conditions.

Biphasic fluorescence kinetics are characteristic of antenna size heterogeneity amongst PS II centres, with the slow phase of fluorescence arising from PS II_β centres and the more rapid phase arising from PS II_α centres. During acclimation of HL-grown cells to LL an increase in the proportion of centres which were PS II_α was observed. Changes in the relative proportion of PS II_β centres during acclimation is shown in Fig. 5c (triangles), which illustrates that a relatively slow shift

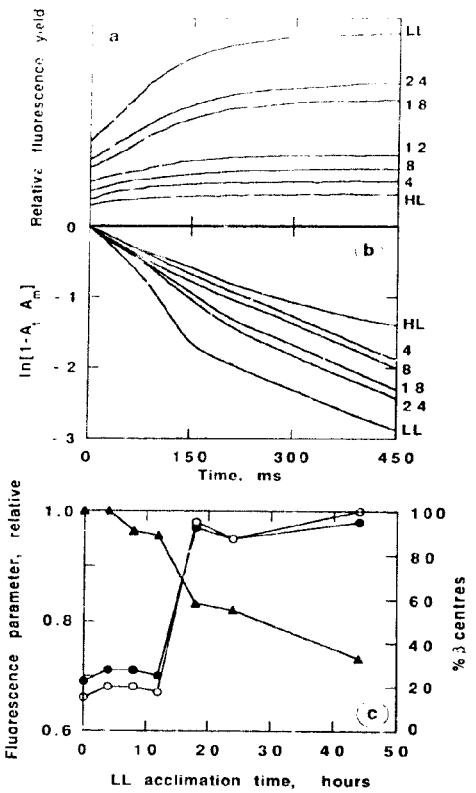

Fig. 5. (a) Room-temperature fluorescence induction in the presence of DCMU from cells of *D. salina* grown under HL and acclimated to LL for varying times indicated. Total chlorophyll concentration was 10 μg ml^{-1}. (b) Semilogarithmic plots of rate of area growth above the fluorescence induction curves shown in (a). The intercept on the y-axis, obtained by linear extrapolation of the slow kinetic component, defines the proportion of PS II$_\beta$ centres in the thylakoid membrane. (c) Changes in the relative values of fluorescence parameters F_m/F_0 (●) and F_v/F_m (○) during LL acclimation of HL-grown *D. salina*. Values derived from the fluorescence induction curves in (a) were normalised to the value F_v/F_m for LL cells. Changes in the proportion of PS II$_\alpha$ centres in the thylakoid membrane, deduced from the kinetic analyses in (b), are also shown (▲).

from PS II$_\alpha$ to PS II$_\beta$ centres over the initial 12 h of the LL acclimation period, followed by a pronounced and rapid shift to PS II$_\alpha$ between 12 and 18 h after the commencement of LL acclimation. The rate of photon utilization by PS II$_\beta$ centres was consistently in the range 3.9–4.1 photons s^{-1}, whereas rates for PS II$_\alpha$ centres increased from 5.1 photons s^{-1} to 9.0 photons s^{-1} over the LL acclimation time-course, suggesting a progressive increase in PS II antenna size from the time-point after which the shift from PS II$_\beta$ to PS II$_\alpha$ began. PS II$_\beta$ centres comprised 32% of the total PS II centres in cells fully acclimated to LL, in agreement with previously reported values for *D. salina* cells grown under normal irradiance [14]. HL-grown cells also exhibited biphasic fluorescence kinetics (Fig. 5b), although these kinetics comprised two relatively slow components and no rapid phase attributable to PS II$_\alpha$ centres. The two slow phases are defined by rate constants for light absorption of 4.0 photons s^{-1} and 2.9 photons s^{-1}. Deconvolution of the fluorescence kinetics indicate that the slower rate is attributable to light absorption by 90% of the total PS II centres in the thylakoid membrane of HL cells. These centres correspond to the PS II$_\gamma$ centres which predominate in HL cells and which have a functional antenna size of only 60 chlorophyll molecules [10]. The rate of light absorption of the remaining 10% of PS II centres in HL thylakoid membranes characterises them as PS II$_\beta$ centres. The very slow kinetic component was no longer evident after 4 h of LL acclimation. HL-cells acclimated to LL for 4 h showed only monophasic fluorescence kinetics characteristic of PS II$_\beta$ centres, indicating rapid conversion of PS II$_\gamma$ to PS II$_\beta$ during the initial phase of reacclimation.

The pronounced increase in the percentage of PS II$_\alpha$ centres between 12 and 18 h after the commencement of acclimation to LL coincided with an elevation in variable fluorescence yield (Fig. 5a) and a pronounced rise in the ratios F_m/F_0 (Fig. 5c, closed circles) and F_v/F_m (Fig. 5c, open circles), supporting the conclusion of increased PS II antenna size and suggesting an increase in quantum yield of PS II photochemistry after an initially constant and relatively low level. The values F_v/F_m and F_m/F_0 remained unchanged at their new, elevated values subsequent to the pronounced rise between 12 and 18 h after the commencement of LL acclimation.

Changes in the polypeptide composition of the thylakoid membrane during acclimation of HL-grown *D. salina* to LL were investigated by SDS-PAGE and immunoblotting. Fig. 6a shows SDS-PAGE anaiysis of polypeptides from thylakoid membranes isolated from cells removed to LL, with each gel lane loaded with thylakoid membrane protein equivalent to 10 μg chlorophyll. No appreciable change in polypeptide composition, specifically any increase in LHC-II polypeptides as a proportion of the total membrane protein complement, was observed until at least 4 h after the commencement of LL acclimation. Subsequent to the initial 4-h acclimation period there was a progressive increase in LHC-II polypeptides as a proportion of the total thylakoid membrane proteins. This phenomenon is most apparent after 18 h of LL acclimation: LHC-II polypeptides became the most prominent species in the thylakoid membrane. There were no apparent major increases in LHC-II content between 12 and 18 h, as might be suggested in light of the rapid increase in variable fluorescence yield at that point in the reacclimation time-course, implying a progressive rather than incremental increase in the levels of LHC-II poly-

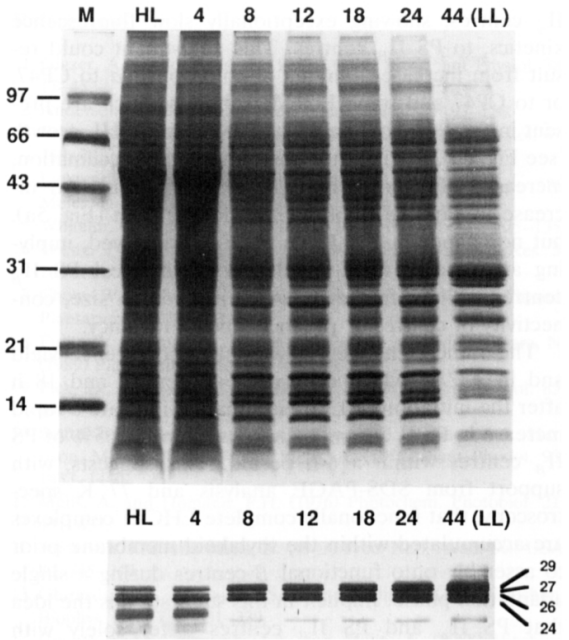

Fig. 6. (a) SDS-PAGE analysis of thylakoid membranes isolated from *D. salina* cells grown under HL and reacclimated to LL for the specified times. Lanes were loaded with material equivalent to 10 μg total chlorophyll. Molecular masses of marker proteins (M) are indicated. LHC-II polypeptides are prominent in the mass range 25–32 kDa. (b) Immunoblot analysis of the LHC-II polypeptides in thylakoid membranes of HL-cells acclimating to LL. Note the absence of changes during the initial 4 h of reacclimation and the subsequent increase in the relative abundance of LHC-II.

peptides incorporated into the thylakoid membrane after the initial 4-h lag period. Immunoblotting with polyclonal antibody to LHC-II (Fig. 6b) emphasises this increase in levels of LHC-II polypeptides during LL acclimation: after an initial four hour lag period, in which no changes in polypeptide composition occurred, there was a progressive increase in the levels of LHC-II polypeptides. The immunoblots also indicate there were no changes in the relative stoichiometries of the LHC-II subunits during LL acclimation. All four LHC-II subunits recognised by the antibody which were present in LL cells were also present in HL cells in the same proportions, albeit at reduced overall levels. This contrasts with observations made with other algal species, in which varying growth irradiance induced variations in both the polypeptide composition and overall size of the PS II antenna [26].

Discussion

Cells of *D. salina* grown under high irradiance show an elevated Chl *a*/*b* ratio symptomatic of a decreased complement of LHCs in the thylakoid membrane, with concomitantly decreased functional antenna size for both photosystems [10]. Removal of HL-grown cells to a LL regime stimulated a decrease in Chl *a*/*b* ratio and induced a temporal sequence of events representing changes in the size and composition of the PS II antenna.

The initial acclimation event gave rise to increased fluorescence emission at 695 nm, which correlated with increased Chl *a*-binding to the CP47 polypeptide of PS II core. This event occurred within 4 h of LL acclimation, and preceded all other significant changes in the PS II antenna. No change in ratio of fluorescence emission at 683 nm to that at 710 nm was observed during this initial period.

These observations suggest that CP47 may associate with the PS II-RC in the absence of bound chlorophyll. This has significant implications for the organisation of the internal antenna of PS II. Recent work has indicated that CP47 is required for assembly of the PS II core in cyanobacteria [27]. In the case of green algae, it has additionally been demonstrated that CP43 and CP47 are independently assembled in the thylakoid membrane of green algae [28], and that while CP47 may function in the initiation of PS II core assembly, CP43 may play a role in initiating early stages of PS II accessory antenna assembly prior to its association with the photosystem core [28]. It may be reasoned therefore that the binding of chlorophyll *a* to CP43 is essential for its primary function, specifically the formation of a pathway of excitation energy transfer from the peripheral antenna of PS II to the reaction centre. If the primary function of CP47 is in the assembly of PS II, chlorophyll-binding would not be essential for this function. During periods of growth under which chlorophyll synthesis was repressed, such as periods of exposure to very high irradiance [8], a prioritization of chlorophyll integration into thylakoid membrane proteins would occur, with full assembly of CP43 competent for energy transfer taking priority over chlorophyll integration into CP47. Such a hierarchical assembly process has previously been suggested for Chl-*b*-deficient maize mutants [29].

The presence of CP47 is required in order to maintain structural stability of PS II [30–32], and is likely, therefore, to be present in the thylakoid membrane of HL-grown *D. salina* cells with or without bound chlorophyll. It cannot, however, conduct excitation energy from the auxiliary antenna to the PS II reaction centre. This rules out any model for the organisation of the PS II antenna in which CP43 and CP47 operate in series to transfer excitation to the reaction centre. Instead, a model in which the two polypeptides act in parallel is required, with CP43 directly connecting the LHC-II antenna to the PS II-RC, as shown in Fig. 7. This model (Fig. 7) is not incompatible with data indicating a more intimate association of the PS II-RC

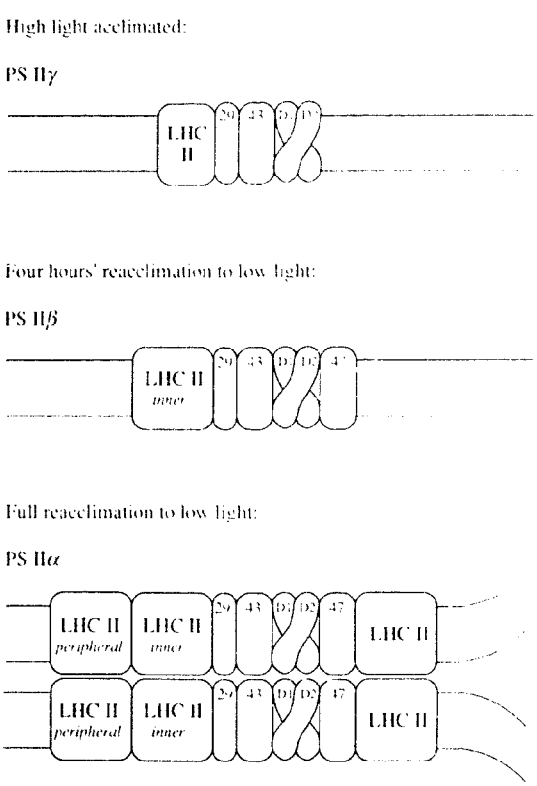

Fig. 7. A model for changes in PS II organisation during acclimation of HL-grown cells to LL, showing additions of CP47 and an inner population of LHC-II to PS II_γ to give PS II_β after 4 h acclimation. Addition of further LHC-IIs (LHC-II peripheral) occurs after full LL acclimation, giving PS II_α centres connected for energy transfer.

with CP47 than with CP43 [33–35]. Solubilization studies do not necessarily suggest close association of CP43 and CP47.

Both the Chl *a*/*b* ratio and the protein/chlorophyll ratio decreased steadily subsequent to the initial 4 h of LL acclimation, suggesting an essentially linear, non-incremental increase in the complement of accessory LHC-II associated with PS II centres in the thylakoid membranes of cells acclimating to LL. Changes in low-temperature fluorescence emission spectra also suggest progressive changes in the organisation of chlorophyll-protein complexes in the thylakoid membrane (Figs. 2 and 3). Removal of the repression of chlorophyll synthesis by transplanting to a low-light intensity regime increases availability of the chlorophylls requisite for LHC-II assembly [8,36] and permits accumulation of the complexes within the thylakoid membrane. The fluorescence induction data (Fig. 5) suggest an adjustment in the PS II antenna during the first 4 h of acclimation which results in a shift from PS II_γ centres, showing exceptionally slow fluorescence kinetics, to PS II_β centres. This adjustment could result from increased binding of chlorophyll *a* to CP47, or to CP47 and some LHC-II subunits which are present in the thylakoid membrane even under HL growth (see Fig. 6b). During the subsequent 8 h of acclimation, increased abundance of LHC-II is apparent. An increase in variable fluorescence yield is seen (Fig. 5a), but no change in F_m/F_0 or F_v/F_m is observed, implying an increase in a population of identical PS II_β centres without further changes in antenna size, connectivity of centres or photochemical efficiency.

The radical changes in variable fluorescence yield and in F_v/F_m which occurred between 12 and 18 h after the initiation of LL reacclimation indicate a rapid increase in PS II antenna size of as many as 65% of PS II_β centres within a 6-h period. This suggests, with support from SDS-PAGE analysis and 77 K spectroscopy, that functionally complete LHC-II complexes are accumulated within the thylakoid membrane prior to assembly onto functional β-centres during a single adaptation phase. Implicit in this suggestion is the idea that PS II_α and PS II_β centres differ solely with respect to their antenna sizes, and that PS II_α centres arise simply as a result of addition of peripheral antenna complexes to PS II_β centres [37].

Addition of LHC-II complexes would also be expected to favour thylakoid membrane stacking, which would account for the greater area of thylakoid appression in LL-acclimated chloroplasts [4,38]. This feature of LL PS II_α membrane organisation is depicted in Fig. 7.

The reacclimating *D. salina* system presents a good model for examining the relationship between subpopulations of PS II centres. If the transition from PS II_β to PS II_α occurs as a consequence of increased antenna size, then the added LHC-II complexes would represent the peripheral antenna which may become phosphorylated [39] during the short-term adaptations to light regime known as State 1–State 2 transitions [40]. Cells of *D. salina* grown under high irradiance and acclimated to low irradiance for 12 h would be expected therefore to be incapable of state transitions. The interaction of short-term, protein phosphorylation-mediated adaptation mechanisms with long-term acclimation phenomena will be discussed in a subsequent paper.

Acknowledgements

This work was supported by the United Kingdom Science and Engineering Research Council in the form of a research studentship (M.A.H), fellowship for visiting overseas professor (A.M.) and research grants (J.F.A.).

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