PS $II_{\alpha/\beta}$ Heterogeneity during the Cell Cycle of the Unicellular Green Alga *Chlorella fusca*

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Fluorescence induction measurements indicate a PS $II_{\alpha/\beta}$ heterogeneity in the unicellular green alga *Chlorella fusca*. Under normal light the relative amount of PS II_α is decreasing from 63% to 43% during the first 8 h of the cell cycle. The rate constant k_α shows a concomitant decrease by 20% in the first 4 h. The relative amount and antenna size of PS II_α is restored in the dark. In contrast, PS II_β-centres show the same rate constant during the whole cell cycle. Under low light k_α is increased by approximately 30% over the cell cycle, whereas no change of k_β is observed.

It is concluded that the PS II organization of *Chlorella* is changed both during the cell cycle and the adaptation to light intensity by a complex regulation (i) involving the antenna size of α-centres and (ii) changing the quantitative relationship of fast and slow reaction centres.

Introduction

The illumination of dark-adapted leaves, isolated chloroplasts, thylakoid membranes or intact green algae induces a complex kinetics of chlorophyll fluorescence [1]. The observable fluorescence induction curve reflects the redox state of the electron acceptor Q of photosystem II [2] and has provided some insight into the structure and function of the photosynthetic apparatus [3]. Every part of the induction curve can be assigned specifically to a section of the photosynthetic electron transport chain (for review see [4]).

After dark adaptation and incubation of chloroplasts or green algae like *Chlorella* with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) a maximum fluorescence level (F_{max}) is reached because DCMU blocks the electron transport at the reducing site of photosystem II (PS II). The kinetics of this fluorescence rise cannot be derived from a single component first order reaction, but the curve rises in a characteristic biphasic manner ([5], see Fig. 1).

In 1975 Melis and Homann [6] derived from this biphasic nature of the fluorescence induction curve of dark-adapted and DCMU-inhibited chloroplasts of higher plants the concept of PS $II_{\alpha/\beta}$ heterogeneity. They proposed two structurally and functionally different types of PS II termed PS II_α and PS II_β. PS II_α is found to have a larger functional antenna size than PS II_β [7] and to be located exclusively in the appressed membrane region, whereas PS II_β is situated in the stroma-exposed part [8]. This concept, however, has been challenged by several authors [9–11].

In addition to higher plants there have been reports on α/β-heterogeneity based on the analysis of fluorescence induction curves in the green alga *Chlorella pyrenoidosa* [12] and in the marine diatom *Phaeodactylum tricornutum* [13]. Senger *et al.* [14] reported variations of the rate constants for the slow and fast reaction centres in *Scenedesmus obliquus*, without a significant change of the PS $II_{\alpha/\beta}$ ratio. Fluorescence decay data of *Chlorella vulgaris* are also in agreement with the assumption of this type of PS II heterogeneity [15].

In this report we show that the relative content of PS II_α and PS II_β and the rate of photon trapping of PS II_α are variable features of PS II organization during the cell cycle of *Chlorella fusca*. These parameters are also changed in a characteristic manner depending on the light intensity applied during cell growth.

Materials and Methods

Cultures of *Chlorella fusca* strain 211-15 from the Collection of Algal Cultures Göttingen, F.R.G., were grown photoautotrophically under "normal"

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(370 $\mu E \cdot m^{-2} \cdot s^{-1}$) and "low" (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) light conditions with Osram L36W/41 "Interna" lamps in combination with Osram L36W/11 "Daylight" (Osram, West-Berlin) at 28 °C in a medium according to Grimme and Boardman [16]. When cells reached a density of 10^6/ml they were synchronized under a light/dark regime of 14:10 h. The cell number and degree of synchrony was determinded with a Coulter Counter (Coulter Instruments, Harpenden, Great Britain). The synchronization index was calculated to be about 0.8 [17].

For fluorescence experiments, aliquots were taken directly from the culture medium at defined times during the cell cycle and measured without dilution. Because of chlorophyll synthesis in growing cells, the chlorophyll concentration in the cuvette was 0.2 to 3.5 μmol/l. This variation of the chlorophyll content did not influence the course of the induction curve. Chlorophyll determination was performed according to Böger [18].

Fluorescence induction measurements were taken with an Aminco DW2 split-beam spectrophotometer (Aminco, Silver Spring, MD, U.S.A.) equipped with a cross illumination device. Green actinic excitation ($\lambda_{max} = 546$ nm, 5.5 Wm^{-2}) was provided by a SFK 11 filter (Schott, Mainz, F.R.G.) to ensure light to be absorbed as equal as possible by both chlorophyll *a* and *b* [7]. The shutter had an opening time of 1–2 ms. The photomultiplier was protected by a cut-off glass filter RG 665 (Schott, Mainz, F.R.G.).

Fluorescence of whole cells was induced after 30 min dark adaptation and a DCMU incubation of 5 min, which ensured a constant F_{max}-plateau (final DCMU concentration 50 μmol/l). Determinations of F_{max} have to be done very carefully in case of the examination of PS II heterogeneity [19]. The induction curves were recorded by a storage oscilloscope (Nicolet Instruments Corporation, Model 200). The area above the fluorescence induction curve was calculated in order to determine the relative amounts of $PS\ II_\alpha$ and $PS\ II_\beta$ and both rate constants [6, 20].

Results

Chlorella fusca shows a similar biphasic nature of its fluorescence induction curve as chloroplasts of higher plants (Fig. 1). A semilogarithmic analysis of the area growth above the induction curve reveals a non-linear first part and a linear second part (Fig. 2). That implies a heterogeneity of PS II, which has been expressed as the two forms, α and β, of reaction centres [6].

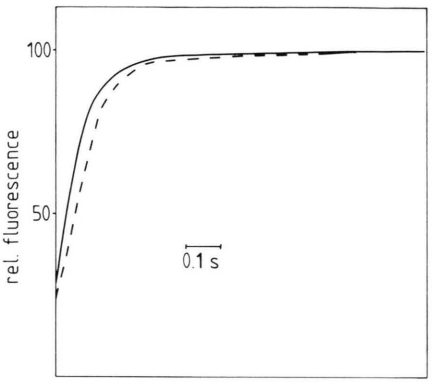

Fig. 1. Fluorescence induction curve of DCMU-inhibited *Chlorella fusca* autospores after 30 min dark adaptation and 5 min incubation with DCMU (50 μmol/l). F_{max} was normalized to 100 relative units. Solid line (——) represents low light and broken line (– – –) normal light growth conditions. Actinic light intensity was 5.5 W/m^2 and chlorophyll content was 0.2 and 0.6 μmol/l, respectively, with a cell number of 10^6 cells per ml. The higher value of F_0 in low light cultures reflects changes in LHC not functionally bound to PS II.

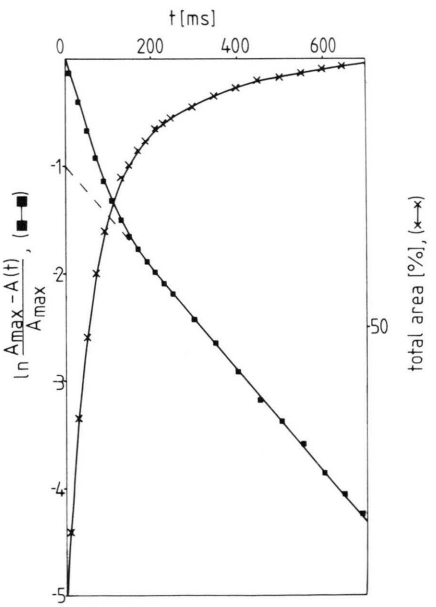

Fig. 2. Area growth above the fluorescence induction curve of autospores of *Chlorella fusca* synchronized under normal light intensity and first order analysis, indicating the biphasic nature of fluorescence induction kinetics.

Under normal light conditions the content of PS II_α-centres in autospores of *Chlorella fusca* is higher than that of the PS II_β-centres (Fig. 3). After onset of light at the beginning of the cell cycle the relative concentration of PS II_α represents 63% and decreases to a minimum of 43% in 8 h cells and remains constant up to the twelfth hour. At the end of the light phase the PS II_α-content is increasing and reaches finally the original level of 63%. The rate constant k_β remains constant with a value of approximately 4.8 s^{-1} all over the cell cycle of *Chlorella* (Fig. 4). Since k is a direct measure for the relative amount of light harvesting pigments which are functionally associated with PS II [7] the absorption cross section of PS II_β does not change in *Chlorella* during the cell cycle under normal light conditions. In contrast to β-centres the rate of photon trapping of α-centres is decreasing during the first 4 h in the light phase from 12.2 to 9.9 s^{-1}. Later on k_α does not change significantly in the light, but reaches the value of autospores in the dark phase, indicating large structural changes associated with PS II_α during the cell cycle of *Chlorella*. In autospores k_α is 2.7 times larger than k_β, and after 8 h in the light the difference is only twofold.

A separate first order analysis of the area above the fluorescence induction curve for PS II_α and PS II_β reveals a constant k_β and a progressively (with induction time) increasing k_α (Fig. 5). A constant value for

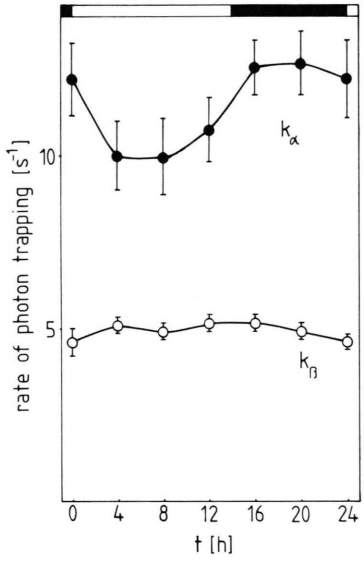

Fig. 4. Rate constants of the slow (k_β) and fast (k_α) part of fluorescence induction curves during the cell cycle of *Chlorella fusca* grown under normal light conditions (n = 13–17, S.D.: k_β ±4–9%, k_α ±8–11%).

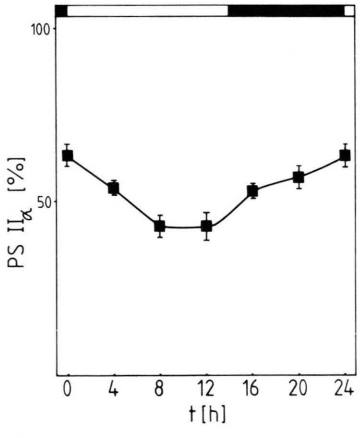

Fig. 3. Relative amount of PS II_α-centres during the light/dark synchronized cell cycle of *Chlorella fusca* grown under normal light conditions (n = 13–17, S.D. ±4–8%). The white bar represents the light phase, the black bar darkness.

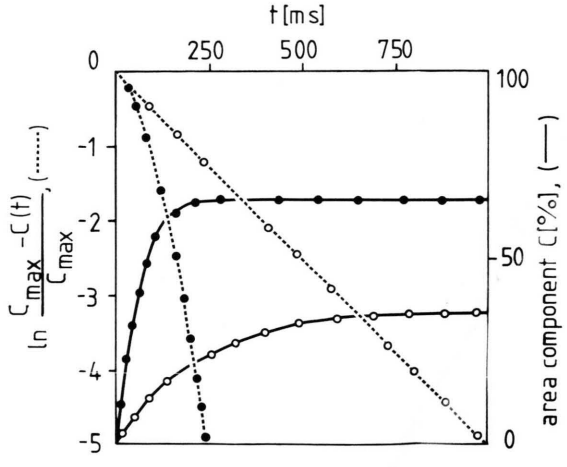

Fig. 5. Area growth above the fluorescence induction curve (solid line) and kinetic analysis (broken line) of the slow β-part (○) and the fast α-part (●) of autospores of *Chlorella fusca* grown under low light.

k_β is indicative for disconnected PS II_β-centres in terms of excitation energy transfer [21]. Non-linearity in a semilogarithmic plot of the photoreduction of PS II_α is interpreted as the formation of aggregates or clusters of those types of photosystems, which share the same statistical pigment bed [7]. The ratio of k_α at the beginning of actinic illumination, when all PS II_α-centers are open, to the terminal value is about 5 for autospores (Fig. 5) and does neither change during the cell cycle nor with light intensity, *i.e.* five PS II_α units form a functional cluster in *Chlorella*.

The rise of the fluorescence induction curve of autospores grown under low light conditions is steeper than its counterpart of normal light cultures (Fig. 1). The reason for the different time course of fluorescence in DCMU-poisoned autospores must be a different organization of PS II_α. Although the relative concentration of PS II_α (and with it also of PS II_β) in autospores does not change with light intensity during synchronization, the rate of photon trapping for PS II_α is 30% larger under low light (Table I). In low light *Chlorella* the antenna size of PS II_α is about 3.6 times larger than that of PS II_β. Ley and Mauzerall [22] stated, that PS II antenna size distribution cannot exceed a factor of about 3 (which holds in our experiments only for normal light conditions). Our methodological approach, differentiating between two different kinetic types of PS II, may explain the difference to these results, which were obtained by absolute measurements of optical cross-sections for photon absorption of PS II [22]. We do not observe changes in k_β, neither during the cell cycle nor with different light intensity. Therefore the effective absorption cross-section of PS II_α enlarges when light intensity decreases while the amount of PS II_β functional antenna pigments appears to be independent of light intensity. The shift in the relative contribution of α- and β-centres during 8 h under low light is similar to normal light, but less pronounced (Table I).

Table I. Rate constants of the slow (k_β) and the fast (k_α) part of the fluorescence induction curve and the relative amounts of PS II_α and PS II_β at normal and low light growing conditions, calculated for *Chlorella fusca* at different times (0 and 8 h) of the cell cycle (n = number of experiments).

	Normal light		Low light	
	0 h cells $n = 14$	8 h cells $n = 17$	0 h cells $n = 13$	8 h cells $n = 13$
k_α (s^{-1})	12.2 ± 1.1	9.9 ± 1.1	17.1 ± 0.9	17.1 ± 1.0
k_β (s^{-1})	4.6 ± 0.4	4.9 ± 0.2	4.8 ± 0.2	5.0 ± 0.3
PS II_α (%)	63 ± 3	43 ± 3	64 ± 2	54 ± 3
PS II_β (%)	37 ± 3	57 ± 3	36 ± 2	46 ± 3

Discussion

Unlike in higher plants the thylakoids in green algae cannot clearly be differentiated into appressed grana and non-appressed stroma regions [23]. But the existence of grana seems no prerequisite for the formation of α-centres in higher plants [20]. Even cyanobacteria without grana show a sigmoidicity in the fluorescence induction curve [24]. In *Chlorella* the quantification of this induction curve reveals a decrease of the relative content of PS II_α-centres from 63% to 43% during the first 8 h of the cell cycle (Fig. 3). The original level of PS II_α-centres, however, is reestablished in the dark. These changes correlate well with the degree of thylakoid banding during illumination, when 2–3 thylakoids form one band, whereas in the dark a band consists of 4–5 membranes [25].

The increasing relative content of β-centres cannot be explained by a preferential synthesis of this PS II-type, although there is an immediate synthesis of reaction centres of PS II [26]. But molecular genetic studies on the accumulation of mRNA of the light-harvesting complex (LHC) in *Chlamydomonas reinhardtii* indicate a delay of LHC synthesis [27], as well as the analysis of pigment proteins [28] and chlorophyll *b* synthesis in *Chlorella* [29], which is mainly associated with LHC [30]. Concluding from our data (Fig. 3, 4) the antenna of newly synthesized β-centres have to be provided by "mobile" LHC. Indeed it has been shown that LHC in higher plants become mobile upon phosphorylation and move from appressed to unstacked membranes. One fourth of LHC may be phosphorylated [31], which correlates well with the loss of 20% in antenna size of PS II_α (Fig. 4).

Low temperature fluorescence studies revealed an antenna net of PS II not properly organized in the first 4 h of the cell cycle [32], the same time, in which k_α is decreasing. Since the smaller antenna of PS II_β does not vary in size, the decreasing average PS II unit size [32] reflects only changes in PS II_α. The

ratio of variable to dead fluorescence (F_{var}/F_0) was taken as an indicator for the coupling of antenna chlorophyll and PS II centres [26]. We did not detect changes in F_{var}/F_0 at room temperature during the cell cycle (data not shown), so that the loss of antenna pigments from PS II_α did not result in LHC incapable of transfering excitation energy to reaction centres. Models of PS II organization during state transitions of the alga *Scenedesmus obliquus* argue indeed on a tight coupling of LHC and the core antenna of α- and β-centres [33].

The idea of a small and invariable antenna size of PS II_β is in good agreement with the result of freeze-fracture studies. Accordingly, the larger and more variable EFs particles are structural equivalents of PS II_α-units and the smaller EFu particles are seen as the more uniformly sized PS II_β-units [23]. In contrast to our results the analysis of fluorescence induction curves and picosecond fluorescence spectroscopy of *Scenedesmus* yielded a constant value for k_α and a change of k_β together with small variations of the relative amount of β-centres [33, 34].

Our explanation concerning the relationship between fast and slow PS II types in the early stages of a developing photosynthetic apparatus in green algae is different from models for higher plants. Although present from the beginning in bean leaves, α- and β-centres develop independently with fast changes of their rate constants, due to fill up of open space by newly synthesized chlorophylls [20]. In another approach addition of peripheral LHC to PS II_β-units will result in the formation of PS II_α-centres and grana partitions [35, 36].

Under low light conditions autospores show a α/β-ratio similar to normal light, but after 8 h there are more α- than β-centres (Table I). The same phenomenon has been observed in low light pea chloroplasts, which is in line with the larger appressed/non-appressed ratio of the thylakoid membranes [37]. The amount of LHC associated with the fast reaction centres under low light is approximately one third higher than under normal light (Table I). This correlates with the higher content of LHC in such algae [30]. In *Chlorella pyrenoidosa* the ratio of reaction centres of PS II and I is at best slightly higher under low light [38]. So we can conclude, that the increased chlorophyll content of *Chlorella* grown under low light [39] is due to an larger antenna array specifically of PS II_α.

Our data on the relative amount of chlorophylls in pigment proteins derived from autospores of normal and low light *Chlorella* [30] do not confirm the conclusion of Percival *et al.* [10], that the PS II/LHC-ratio (measured as Chl *a*/*b*-ratio), is the major determinant of the relative contribution of PS II_α and PS II_β. Although we found a considerable decrease in the PS II/LHC-ratio in low light autospores [30], there has been no change in the relative amount of slow and fast PS II reaction centres (Table I).

The antenna size of PS II_β is independent of light intensity, so that PS II_β may represent the smallest PS II unit *in vivo* even under different environmental conditions or at different developmental stages, consisting of a core complex with few tightly bound antenna pigments. A similar suggestion has been made on the basis of radiation inactivation of PS II [40].

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